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THE EFFECTS OF ACUTE BLUNT TRAUMA AND THERAPY ON THE ULTRASTRUCTURE AND PROTEIN CONTENT OF SKELETAL MUSCLE

by

©

BRIAN DAVID FISHER

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSICAL EDUCATION
AND SPORTS STUDIES

EDMONTON, ALBERTA

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE EFFECTS OF ACUTE BLUNT TRAUMA AND THERAPY ON THE ULTRASTRUCTURE AND PROTEIN CONTENT OF SKELETAL MUSCLE submitted by BRIAN DAVID FISHER in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in PHYSICAL EDUCATION AND SPORT STUDIES

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ABSTRACT

The focus of this research was to investigate the effects of acute blunt trauma, drug and physical therapies on the ultrastructure and protein content of skeletal muscle. A simple device for producing a humane and reproducible experimental model of blunt trauma to rat skeletal muscle was used. Ultrastructural events in the traumatized muscle were observed over 30 days. From 0 to 2 days after trauma there was a marked loss of the muscle, associated with an acute inflammatory response. From 3 to 14 days, the muscle underwent regeneration with a rapid proliferation of sarcolemmal nuclei, activation of satellite cells, and restoration of sarcomeres. Muscle regeneration was complete 21 days following trauma. By three days post-trauma, muscle protein decreased (40-58%) compared to muscles of control rats ($P>0.05$). The protein content of the uninjured contralateral muscles decreased an average of 23% when compared to muscles of uninjured controls ($P>0.05$). Twenty-one days were required for the protein contents of the injured muscles to return to those of the normal control muscles. The administration of naproxen, a nonsteroidal anti-inflammatory drug (NSAID), partially prevented the decrease in protein contents of the traumatized muscles. The positive effect of naproxen appeared to be related to a prostaglandin-dependent mechanism.

The effect of ultrasound therapy on the protein content of muscles that were traumatized or in the vicinity of trauma, varied with muscle type. There was no significant difference in protein content between the traumatized medial gastrocnemius muscle that was treated with ultrasound between days 3 and 9 after injury, and the untreated trauma control. The traumatized soleus and lateral gastrocnemius muscles showed increases in their protein contents as a result of ultrasound therapy when compared to their corresponding trauma controls. Ultrasound therapy decreased PGE_2 production in the traumatized muscles when compared to the corresponding trauma-control muscles.

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CHAPTER I: OVERVIEW OF THE PROBLEM

Introduction

The past 15-20 years represent an era of prodigious growth for sports and fitness related activities. Unfortunately, increased participation has contributed to a rise in the number of sports-related injuries (Adams et al. 1987). The majority of these involve skeletal muscle and range from minute separation of the connective tissue and muscle fibre to a complete tendinous avulsion or muscle rupture (Reilly 1981). Because knowledge of muscle injuries is limited, the diagnosis and treatment of these conditions are a challenge to sports medicine physicians and therapists. Further, similar types of injuries are subjected to different treatment methods due to the lack of integrated scientifically prescribed treatment (Jackson 1978). Clinicians are forced to rely on their own trial and error methods of treatment because of inadaquate attention to and availability of the basic research required to evaluate various treatment modalities. To gain the greatest benefit from our therapies we require a basic understanding of the body's pathophysiological response to skeletal muscle trauma and the physiological response to the therapy.

The main purpose of this thesis is: (1) to develop a new experimental model that produces a standardized muscle injury and allows the investigator to follow the morphological and biochemical changes which take place during the degenerative and regenerative phases of muscle injury and repair, (2) to examine the efficacy of non-steroidal anti-inflammatory drugs and ultrasound on prostaglandin production and protein content of the muscle following injury.

Sports medicine practitioners working in the field of sports medicine have a wide variety of drugs and other forms of therapy at their disposal for the treatment of acute inflammation secondary to muscle injury and for the promotion of muscle regeneration. These include the corticosteroids, muscle relaxants, non-steroidal anti-inflammatory drugs (NSAID) and ultrasound (Vane 1971, Salminen & Kihlstrom 1986, Hashishe et al. 1986, Stratton et al. 1984).

The nonsteroidal anti-inflammatory drugs act by inhibiting the biosynthesis of prostaglandins. The recent work of several authors indicates that prostaglandin E_2 (PGE_2) induces changes in the regulation of proteolysis following several physical and pathological states (Rodemann & Goldberg 1982, Baracos et al. 1983). More important to sport, is the possible relationship of an increased production of PGE_2 following injury and a further increase in proteolysis when compared to the non-injured state. Although numerous studies have shown that prostaglandins are increased following injury to soft tissue (Davies et al. 1984, Vane 1971, Goldstein et al. 1977), few studies, if any, have investigated the effects of acute blunt trauma and prostaglandin production on protein content in muscle. Recently, however, prostaglandins have been shown to play a role in muscle protein turnover (Reeds & Palmer, 1984). Specifically prostaglandin E_2 is thought to have a catabolic effect on muscle tissue.

Ultrasound is a commonly used modality (Fyfe & Chahl, 1985). Recent studies indicate that the therapeutic benefit derived from ultrasound is in reducing the inflammatory phase (Fyfe & Bullock 1985,Fyfe and Chahl 1984), others report that it has a placebo effect (Hashishe et al. 1986) and some claim it has no effect on inflammation. (Goddard et al. 1983). Finally, Stratton et al. (1984) examined the effects of ultrasound on traumatized muscle and reported that higher intensities of ultrasound (0.75 vs 1.5 watts/cm^2) produce a greater healing effect in injured muscle. Thus, the effects of these therapies have not been investigated adequately and some controversy exists as to their efficacy.

LITERATURE REVIEW

The purpose of this literature review is to clarify and detail: a) current understanding of the response of muscle to injury, b) the regeneration and repair of the muscle following acute blunt trauma, c) and to survey sports therapies and concepts about the modes of action of these therapies.

A. EXPERIMENTAL MODELS OF MUSCLE INJURY

i. Crush/Tear Injury

A wide variety of experimental models for the study of muscle regeneration has been employed to produce trauma in skeletal muscle (Table 1). Some models, such as crush or tearing, were chosen because they closely resemble traumatic situations similar to which muscle may be exposed in sports. These techniques allowed the investigator to follow the degenerative and regenerative phases of the muscle (Allbrook & Baker 1966).

Early studies using the crush technique were carried out using surgical clamps or forceps and simply crushing the muscle between the two jaws. This technique was reported to be useful because at the site of injury, all fibres were found to be damaged while still being intact and maintaining their integrity and alignment (Allbrook & Baker 1966, LeGross Clark & Blomfield 1945, Jarvinen & Sorvari 1975, Bischoff 1975, Schultz et al. 1986). Others used a hammer device which delivered single or multiple blows to the muscle (Allbrook and Baker 1966, Tischler et al. 1982). Allbrook & Baker (1966), while studying the injured limb syndrome, employed a device which delivered a single blow to the limb of such magnitude that it not only injured the muscle but fractured the bone. In contrast, Tischler et al. (1982) studied the effects of light multiple blows with a reflex hammer to the muscle over an extended period of time. This investigator reported similar ultrastructural changes occuring

Table 1. Experimental Procedures for the Study of Muscle Injury and Regeneration

TYPE OF INJURY	AUTHOR	COMMENT
CRUSH		
	Jarvinen M, Sorvari T. 1975	Blunt Trauma
	Gutman E, Carlson B. 1977	Clamp
	Millar G, 1974	Crush
	Allbrook D 1962	Crush
	Allbrook D 1966	Hammer Device
	Schultz H, Jaryszak D 1985	Clamp
EXERCISE		
	Myllyla R, Salminen A 1986	Running
	Salminen A, 1984	Running
	Armstrong R, Ogilvie R 1983	Eccentric
	Giddings C, Neaves W 1985	Weight-Lifting
	Armstrong R, 1986	Review
	Hoppeler H, 1986	Review
ISCHEMIA		
	Le Gros Clark W, Blomfield L 1945	Devascularized
	Karpati G, Carpenter S, 1974	Denervation
	Santavirta S, Luoma S,1979	Tourniquet
	Makitie J, Teravainen H, 1977	Tempory Ischemia
	Hall-Craggs E, 1978	Ligation

INJECTION	AUTHOR	COMMENT
	Basson M, Carlson B 1980	Carbocain
	Mussini M, Favaro D, 1987	Bupivacaine
	Benoit P, Belt W, 1970	Bupivacaine
	Dolwick M, et al. 1977	Lidocaine
	Foster A, Carlson B, 1980	Bupivacaine
	Maltin C, et al. 1983	Snake-venom
	Ownby C, Odell G, 1983	Tarantula venom
	Queiroz L, et al. 1984	Snake-venom
	Tu A, 1983	Pit Viper venoms
MINCING		
	Studitsky A, 1959	Transplantation
	Carlson B, 1972	Minced
	Carlson B, Gutmann E 1972	Minced
MICROPUNCTURE	Karpati G, Carpenter S, 1982	Large bore needle
STRAIN INJURY		
	Panteles K Nikolaou M 1987	Forced Lengthing
	Garrett w, et al. 1987	Forced Lengthing

Various experimental proceedures producing muscle injury for the study of muscle regeneration.

in the muscle as produced with a single blow. Tearing or straining the muscle to failure is another technique which has been employed to study muscle injury (Nikolaou et al. 1987, Garrett et al. 1987). The muscle was rapidly lengthened (stretched) to failure or passively stretched to the point of muscle tear. Following injury, the muscle was examined at different time points to determine the force generating capacity of the muscle. A notable finding in Garrett's (1987) study was that muscles that were contracted at the time of injury received less damage and healed faster than passively injured muscles. Further studies using similar techniques of strain to muscle failure report that the injured muscle begins functional recovery by 48 hours despite inflammation and the regeneration process (Nikolaou et al. 1987).

ii. Local Drug Administration

The injection of local anaesthetics offer an alternate technique to crush or tear when examining muscle injury. Yagielu & Benoit (1981) investigated samples of human muscle after the injection of lidocaine and epinephrine and reported degenerative and regenerative changes similar to those observed following crush or tear. Snake or spider venoms also produce muscle damage. In this case, the injury is widespread and acts rapidly on the connective tissue of the muscle sparing only the plasma membrane and the satellite cells (Ownby et al. 1983).

iii. Ischemia

Temporary ischemia, caused by either direct vascular ligation or tourniquet, is another method of producing muscle degeneration and regeneration (Carpenter & Karpati 1984, Santavista et al. 1979). The point of using this technique to study degeneration and regeneration in a hypoxic enviroment is in injuries where the vascular supply is damaged.

iv. Exercise-Induced Injury

Recently, several investigations have reported on the effects of exercise damage on muscle (Armstrong & Ogilvie 1983, Friden & Ostrom 1983, Hoppeler 1986, Warhol et al. 1985). Biochemical and morphological studies indicate that local muscle damage occurs as a result of an overload to the metabolic system, whereby the demand for ATP in the fibre exceeds ATP production, or mechanical strain such as stretching the fibre beyond its normal range (Armstrong & Ogilvie 1983). Muscle injury may also result from mechanical events such as eccentric exercise (Friden & Ostrom 1983). Thus, when rats ran downhill on a treadmill for 90 minutes, ultrastructural changes were observed throughout the entire muscle when examined under the light microscope (Hoppeler 1986). Similar disruptions have been reported in humans after exhaustive eccentric exercise (Armstrong & Ogilvie 1983). In addition to histological and ultrastructural evidence, studies in rodents subjected to eccentric exercise showed increases in the activity of various lysosomal enzymes which serve to degrade cellular proteins (Warhol et al. 1985).

v. Summary

It is essential to understand the recovery process governing muscle following injury. Moreover, the fundamental catabolic events during the degenerative phase serve as a framework for examining the collective stages of the regenerative process. Numerous experimental models have been employed to investigate muscle regeneration (Carpenter & Karpati 1984). Some were designed because they closely duplicated traumatic situations common in daily events and others because of their potential analytical value (Carlson 1976). In this section of the chapter, I have attempted to review injury models which produce events similar to those observed following trauma. And although they may be similar in morphological outcome, only a few have attempted to examine skeletal muscle following a single blow (Allbrook & Baker 1966, Stratton et al. 1984). Blunt trauma resulting in a crush would appear to be a major cause of muscle damage in sport (Reilly 1981). I have therefore elected to study skeletal muscle response to acute blunt trauma using a device which delivers a single blow.

B. THE ACUTE INFLAMMATORY RESPONSE

The activation of the acute inflammatory response following injury involves the amplification and propagation of cells of the reticuloendothelial system (Rubin & Faber 1988). The initial events occur within the microvasculature at the level of the capillary and post-capillary venule. Found within this vascular network are the major components of the acute inflammatory reaction, including, basophils, platelets, red blood cells, plasma, and circulating monocytes. These components are normally contained within the intravascular compartment by a continuous layer of endothelium which is joined by tight junctions and separated from the tissue by a limiting basement membrane (Rubin & Farber 1988). Following trauma, the structure of the vascular wall changes, producing a loss of endothelial cell integrity, an escape of plasma and fluid from the intravascular compartment and the release of both white and red cell extravasation into the extravascular space (Gallin et al. 1986).

Following trauma, specific inflammatory mediators are produced at the injury site and these regulate the calibre and permeability of blood vessels in the region (Wollf 1986). Of these mediators, vasoactive molecules such as histamine and prostaglandins act directly on the vasculature producing increased vascular permeability. Chemotactic factors, such as mast cell products, (see table 2) are released and recruit white blood cells from the vasculature into the injury site (Gallin et al. 1986). Once at the

Table 2 MEDIATORS AND CELLS OF THE ACUTE INFLAMMATORY RESPONSE

Cell Type	Primary Inflammatory Mediator
Mast Cells and Basophils	histamine leukotrienes prostaglandins platelet activating factor eosinophil activating factor
Macrophages Monocytes	hydrolytic enzymes phospholipase A_2 prostaglandins leukotrienes cytokines e.g. Interleukin-1 oxygen metabolites complement
Platelets	serotonin Ca^{2+} ADP fibrinogen platelet-derived growth factor Thromboxane A_2 Lysosomal hydrolyses
Polymorphonuclear leukocytes	Lysosomal enzymes Phospholipase A_2 proteolytic enzymes oxygen metabolites
Eosinophils	Lysosomal enzymes Oxygen metabolites

injury site, the white blood cells secrete a number of additional inflammatory mediators which augment the acute inflammatory response (Rubin & Farber 1988).

i. Initial vascular response to trauma

The initial vascular response to trauma is a transient vasospasm which is mediated by both chemical and neurogenic mediators and usually resolves within seconds or minutes. This is followed by vasodilatation which is produced by the release of specific vasoactive mediators, such as histamine and serotonin (5-hydroxytryptamine). These vasoactive mediators are derived from both plasma and cellular sources. The imported cellular sources of vasoactive mediators are circulating platelets, tissue mast cells, and basophils (Rubin & Farber 1988).

ii. Mast cells and basophils

Mast cells and basophils were first identified by Paul and Ehrlich over a centry ago. The mast cell is located within the connective tissue frame work whereas the basophil circulates throughout the vasculature. Both cells serve as additional sources for the release of vasoactive mediators (Gallin et al. 1986). Following injury, acute inflammatory mediators such as prostaglandins and activated platelet activating factor trigger mast cells and basophilic cells causing the release of electron-dense cytoplasmic granules into the extra-cellular space (Beaver 1978). These granules contain histamine, acid mucopolysaccharides and chemotactic mediators for eosinophils and neutrophils. Histamine, more importantly, produces increased vascular permeability, vasodilation and pain. Histamine also enhances the production of prostaglandin F_2 from damaged cells (Schulman 1987).

iii. Plasma-derived inflammatory mediators

An additional source for the production of vasoactive mediators is from the plasma. Activation of Hageman factor (Factor XII) by exposure to negatively charged surfaces or to proteolytic enzymes or basement membranes results in the proteolytic activation of additional plasma factors, such as plasmin, the complement pathway and kallikrein (Cochrane 1982). Complement activation following injury results in the production of a series of activation peptides, which increase vascular permeability and cause histamine to be released from the mast cells (Rubin & Farber 1988). Plasma kallikrein produced by activated Hageman factor cleaves high-molecular weight kininogen, thereby producing a number of low-molecular weight peptides, commonly referred to as kinins. The most notable of the foregoing is bradykinin. Bradykinin is a monopeptide which produces changes in the endothelium that lead to edema and the onset of pain (Cochrane 1982).

Additional mediators produced during acute inflammation are derivatives of fatty acids and phospholipids. Following trauma, cells within the injury site release arachidonic acid by one of two metabolic pathways (Vane 1971). The first pathway involves stimulus-induced activation of phospholipase A_2; this in turn enhances the hydrolysis of arachidonic acid from the glycerol backbone of membrane phospholipids (Ferreira 1972). During injury, phospholidylcholine is an important substrate of phospholipase A_2 and serves as the major source of arachidonic acid from the injured cell (Ishizaka, 1981). Another way in which free arachidonic acid is produced is by the metabolism of phospholidylinositol by phospholipase C to diacylglycerol and inositol phosphates. Diacylglycerol lipase then cleaves arachidonic acid from diacylglycerol. The free arachidonic acid can now be metabolized via two pathways: lipoxygenation, thereby producing leukotrienes, and by cyclo-oxygenation and the subsequent generation of thromboxanes and prostaglandins (Kennerly 1979). A more detailed explanation regarding prostaglandin production follows in a later section of this thesis dissertation.

iv. Platelets

During the acute inflammatory phase platelets secrete growth factors, and a variety of acute inflammatory mediators which are vasoactive. Of these histamine, serotonin, Ca^{++} and adenosine diphosphate (ADP); alpha granules containing coagulation proteins, fibrinogen and platelet-derived growth factor are reported to be the most potent (Gallin et al. 1986). When platelets make contact with fibrillar collagen or thrombin they may degranulate, releasing histamine and serotonin which further increase vascular permeability. The arachidonic acid metabolite thromboxane A_2 is also released from platelets (Rubin & Farber 1988).

v. Second phase of acute inflammation

The cellular recruitment or second phase of acute inflammation involves the accumulation of polymorphonuclear leukocytes (PMNs) at the injury site. This phase occurs within 24 hours following trauma and is brought about by locally generated soluble mediators commonly referred to as chemotactic factors (Snyderman 1984a). Chemotactic factors, which recruit additional cell types such as monocytes, basophils, eosinophils and lymphocytes, also are produced within this time period by the damaged tissue (Snyderman 1984b).

vi. Circulating monocytes

The functional response of the circulating monocytes when stimulated by chemotactic signals involves cell margination along the wall of the vessel, adherence of the monocytes to the vascular endothelium, emigration through the wall of the vessel, and finally, migration toward the chemotactic signal (Gallin et al. 1986). Monocyte cell activation is initiated by such events as the binding of chemotactic mediators or antibody-antigen complexes to specific receptors on the membrane of the cell (Snyderman 1984b).

The mononuclear cells which participate in the acute inflammatory response and act as important scavengers following injury are termed macrophages (Partridge 1977). Macrophages take up, detoxify and degrade the damaged tissue (Gallin et al. 1986). The migration of macrophages (monocytes) from the blood stream to the damaged tissue represents one of the most important components of the inflammatory response (Adams 1984). The migration of macrophages is brought about by the activation of the paracrine system. This migration occurs when lymphocytes contact specific antigens (i.e. damaged muscle) and release soluble glycoproteins which attract and activate the macrophage (Mackaness 1970).

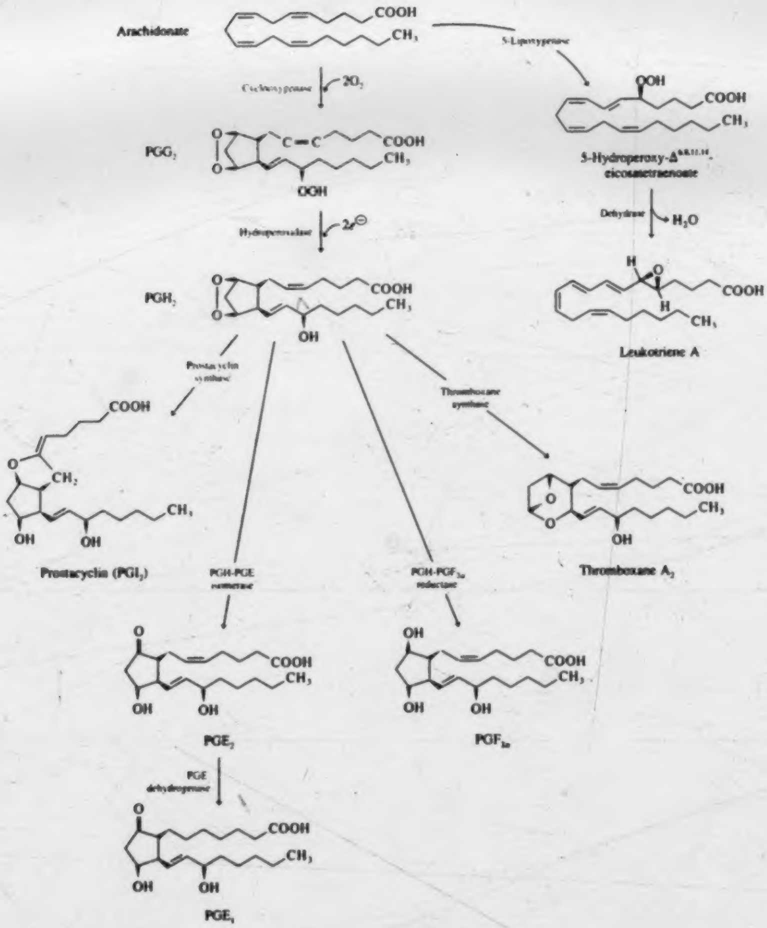
The activated macrophages can now develop in several possible directions which lead to an enhanced ability to orchestrate the acute inflammatory response (Gallin et al. 1986). The activated macrophage is capable of releasing up to 80 different soluble molecular products. The macrophage can secrete a wide variety of acid hydrolases and neutral proteases, several antiproteases, a variety of metabolites of arachidonic acid and various factors which regulate and stimulate the function of other cells (Unanue 1986). In addition to the activation phase, the macrophage also controls the ongoing inflammatory response by regulating the B- and T-lymphocytes and the replication of fibroblasts (Gallin et al. 1986, Rubin & Farber 1988). Finally, another important activity of activated macrophage is the secretion of the metabolites of arachidonic acid. Large amounts of prostaglandins of the E and F series have been detected at the site of injury, and it has been demonstrated that these compounds are synthesised by macrophages (Zurier 1975).

The down-regulation of acute inflammation can be brought about by a number of cellular factors, and is highly complex. For example, neutrophils in vitro, are reported to contain selective lysosomal constituents which can inhibit cyclic 3',5'-adenosine monophosphate (cAMP) directly (Weissman et al. 1975). A key inflammatory mediator is interleukin-1 which in turn acts upon many tissues which results in the increased production of prostaglandins (Rubin & Farber 1988). Prostaglandins of the E series and prostaglandin I_2 also inhibit leukocyte chemotaxis, phagocytosis and the generation of oxygen-derived free radicals (Woolf 1986, Gallin et al. 1986). The function of B and T lymphocytes are also inhibited by prostaglandins of the E series, which suppress the proliferative responses of human lymphocytes to mitogens, antibody production, lymphocyte-mediated cytotoxicity (Rubin & Farber 1988).

vii. Biosynthesis of prostaglandins and their role in inflammation

The oxygenation of arachidonic acid and other polyunsaturated fatty acids leads to the formation of prostaglandins (PG). PG, like other compounds of the arachidonic acid cascade, contribute greatly to the inflammatory response (Ferreira 1973, Vane 1971). Arachidonic acid is an ubiquitous constituent of cell membrane phospholipids, and when liberated to the action of phospholipase, a variety of chemical transformations occur (Goetzly 1981, Figure 1). The first of these is the conversion of free arachidonic acid to unstable prostaglandin endoperoxides (PGH_2,PGG_2) by way of the fatty acid

Figure 1. Pathways for the conversion of arachidonate to prostaglandins, thromboxanes and leukotrienes



Adapted from Rawn (1989)

cyclo-oxygenase pathway. Both PGH_2 and PGG_2 act as potent vasoconstrictors and stimulate irreversible aggregation of platelets (Goldstein 1977). The endoperoxides undergo further transformation resulting in stable prostaglandins (e.g. PGE_2). In the platelet endoperoxides are transformed to thromboxane A_2 which is reported to be a vasoconstrictor and promoter of platelet aggregation (Vane 1971). In the capillary endothelial cell the endoperoxides are transformed into PGI_2, a potent vasodilator and inhibitor of platelet aggregation. Drawing from the above, there appears to be antagonistic roles between thromboxane A_2 and PGI_2 in modulating vascular tone and patency (Moncada 1977).

The formation of prostaglandins is brought about by the release of arachidonic acid from membrane phospholipids by acylhydrolase phospholipase A_2 (Neidleman 1986). This release of arachidonic acid may produce changes in the permeability of the plasma membrane (especially to calcium) and these changes are reported to play an important role in chemotactic signal transduction. Further, arachidonic acid release is followed by guanosyl cyclase activation and this causes the release of cGMP. The cyclic nucleotides have been shown to play an important part in the initiation of movement of phogocytic cells (Woolf 1986).

A number of different enzymes are involved in PG synthesis, and these are referred to as PG synthetase, PG endoperoxide synthetase and cyclo-oxygenase (Figure 1). Of these three, cyclo-oxygenase is reported to be present in all tissue of the body except for red blood cells (Neidleman 1986). As described above, the formation of the primary PG is through non-enzymatic degradation of the endoperoxides; however, the formation of PGE_2 and PGD_2 can be catalysed by PG isomerase. Few reports have shown that $PGF_{2\alpha}$ is catalyzed by an isomerase and $PGF_{2\alpha}$ formed through the reduction of endoperoxide and other reducing agents (Neidleman 1986).

C. OVERVIEW OF MUSCLE REGENERATION FOLLOWING INJURY

Muscle tissue has been reported to have an excellent capacity for repair following injury (Allbrook & Baker 1966, Carlson 1976, Carpenter & Karpati 1984, Engle 1986) . The early events in this process are inflammatory; they are connected with damage control. These have been detailed above, although it should be recognized that the processes of degeneration and regeneration overlap.

Early reports describing muscle pathology and regeneration subdivided muscle regeneration into two separate categories: "continuous" and "discontinous" regeneration. Continous muscle regeneration is brought about when the muscle fibres have been damaged by tear, crush or transected; the ultrastructure is characterized by a regeneration of a basophilic sarcoplasm with nuclei similar in structure to those of the myotube nuclei (Woo & Buckwalker 1977). This is the usual form of muscle fibre regeneration in which a segment of the injured fibre undergoes necrotic degeneration but the sarcolemma remains intact. Discontinous regeneration occurs when the fibre is completely destroyed, then formed entirely de novo with no connection to the pre-existing muscle fibre (Engle 1986, Woo 1977).

Injury to muscle resulting in a crush or tear may be similar to that received during a sporting event. The sarcolemma surrounding the fibre would most likely be spared because the mechanisms involved may not produce excessive stretch or increase the range of motion

(Garrett et al. 1987). However, the traumatized fibres undergo degeneration followed by regeneration (Engle 1986). The process of muscle regeneration in the presence of an intact sarcolemma is a complex series of events involving the infiltration of inflammatory cells in response to mediators (see above), the proliferation of myogenic nuclei and satellite cells, and the formation of myotubes (Engle 1986, Woo & Buckwalker 1977, Carpenter & Karpati 1984). This process is referred to as continuous regeneration and will be emphasized throughout the thesis (Ali 1979, Shafiq & Gorycki 1966).

D. ULTRASTRUCTURAL EVENTS FOLLOWING ACUTE TRAUMA

Following trauma to muscle, the tough connective tissue of the endomysium usually remains intact and muscle degeneration begins within the sarcolemma, followed by disruption of the sarcomeres at the Z-line, swelling of the mitochondria, and pyknosis of the sarcolemmal nuclei (Hudgson & Field 1973). Edema and hemorrhage into the extracellular space increases the extracellular pressure (Carpenter & Karpati 1984). Within a few hours after the injury, polyneutrophils invade the damaged area followed by macrophages which penetrate the sarcolemma and begin to phagocytose the damaged sarcoplasm (Bischoff 1986a). Partridge & Slapen (1977) have suggested that myogenesis cannot take place until macrophages have invaded the damaged area. However, Bischoff et al. (1986), recently have suggested that it is the decrease in the mass of the cytoplasm of the muscle fibre which provides the stimulus for myogenesis.

In addition to the cellular components which arrive from the vascular system and set up the acute inflammatory response to initiate phagocytosis, the muscle also possesses an intrinsic mechanism for the removal of damaged sarcoplasm. This is carried out by lysosomal hydrolases which are capable of completely degrading the muscle fibre (Carpenter & Karpati 1984).

Following acute blunt trauma, the muscle progresses through a number of degenerative and regenerative changes. If the sarcolemma remains intact then ongoing regeneration occurs. Here, a budding or outgrowth takes place from the end of the partly damaged segment (Ali 1979). This budding is characterized by an outgrowth of basophilic sarcoplasm which contains varying numbers of nuclei. The origin of these nuclei cannot be determined; however, possible explanations are: (1) that myonuclei possess the capability to migrate into the budding segment of the muscle, (2) that myoblastic cells can fuse, and (3) that both of the above events take place (Bischoff 1986a). At seven to ten days from the beginning of the regeneration phase, aggregates of myofibrils can be observed in the sarcoplasm, and later these organize into sarcomeres to give rise to muscle fibres of fetal-type (Carpenter & Karpati 1984). Concurrently there is an increase in the number of activated fibroblasts in the endomysium giving rise to scarring or fibrosis (Garrett 1987, Nikolaou 1987). The final events of the process in traumatized muscle depend on the extent of damage to the sarcolemmal membrane at the time of injury, and the integrity of the innervation and the blood supply to the damaged area (Engle 1986).

E. MUSCLE REGENERATION: ULTRASTRUCTURAL EVENTS

Before the topic of muscle regeneration at the ultrastructural level can be approached, it is necessary to establish the nomenclature describing the mesodermal cell population. Myogenic cells have a unique embryological origin which differs from that of other mesodermal cell types (Chevalier & Kieng 1977). A myoblast is defined as a postmitotic mononucleated cell produced during myogenesis at the embryonic stage. A precursor cell of mesodermal origin which is undergoing cell division is referred to as a presumptive myoblast. A satellite cell is a presumptive myoblast, which has been removed from the cell cycle for an indefinite period of time (Bischoff 1986a, Engle 1986). Myoblasts are capable of synthesizing contractile proteins and engaging in cell fusion (Engle 1986). These properties are not shared by presumptive myoblasts (Holtzer et al. 1970). Myoblasts fuse to form long cylindrical, multinucleated cells termed primary myotubes (Carpenter & Karpati 1984). The myotube displays central nucleation surrounded by aggregrates of myofibrils. Alongside the primary myotubes are longitudinally arranged myoblasts, which give rise to secondary myotubes (Kelly & McGrath 1986). The primary and secondary myotubes share the same basal lamina during the early stages of regeneration. As the secondary myotube matures it develops its own basal lamina and becomes a separate fibre. In mature muscle fibres, the sarcolemmal nuclei come to occupy a peripheral location beneath the sarcolemmal membrane (Ontell & Kozeka 1984).

i. Sarcolemmal Nuclei

During regeneration various cellular organelles become activated and involved in repair. The muscle nuclei leave their subsarcolemmal position and migrate to the central region of the fibre. This region of the sarcoplasm becomes rich in rough endoplasmic reticulum, Golgi apparatus and free ribosomes. The nuclear membrane loses its smooth contour and becomes wrinkled and infolded (Moss & Leblond 1971). As regeneration progresses the nuclei enlarge and become pale. This pallor is indicative of transcriptional activity and persists until the muscle fibre has fully regenerated (Vassilopoulos 1976).

ii. Satellite Cells

The spindle-shaped satellite cells differ from myoblasts in that they take a position peripheral to the growing myotubes and are covered by the basal lamina (Engle 1986, Bischoff et al. 1986). Sattelite cells migrate to a damaged region and may:

1. continue to divide and add to the further maintenance of the peripheral cell population,
2. fuse with the differentiating and maturing myofibres,
3. maintain their autonomy and serve as part of the stem cell pool which is activated following injury

(Bischoff et al. 1975). It is to this third type of activity that I will refer again when reviewing muscle regeneration.

The satellite cell is a flattened mononuclear cell with a dark nucleus found beneath the basal lamina. In the neonate, these cells are numerous and subsequently decline with age (Allbrook & Baker 1966). Slow twitch fibres differ substantially in their content of satellite cells when compared to fast twitch fibres. Gibson & Schultz (1983) reported that both the proportion and absolute number of satellite cells to myonuclei are greater in the slow soleus muscle than in the fast extensor digitorum muscle of the rat. In the young growing rat, the satellite cells synthesize DNA and divide. After division, some still remain as satellite cells and others fuse with the muscle fibre to increase its population of nuclei (Moss & Leblond 1971). As the muscle fibre matures, the satellite cells decrease their myogenic activity and maintain an independent position against the plasma membrane and apart from the muscle fibre. During the necrotic phase following muscle injury, the satellite cells become activated and contribute myoblasts which repair the necrotic segment (Schmalbruch & Hellhammer 1976). Snow (1977), using direct in vivo techniques, demonstrated that satellite cells are precursors for the myoblast. This was accomplished by exposing the muscle to H-thymidine so that only the nuclei of satellite cells would become labeled. The investigators observed the regeneration of injured muscle during the formation of new myotubes. The results revealed that regenerating fibres arising from injured muscle fibres contained myonuclei that were not labeled. However, the injured muscle fibres that contained labeled satellite cells produced regenerated nuclei with the radioactive label. Other in vitro studies have confirmed that satellite cells are capable of proliferation and can fuse with myotubes (Bischoff 1986a).

Satellite cells can be present at the site of injury or can migrate from an uninjured section of the muscle fibre. The signal that activates satellite cells is unknown; activation may occur as a result of the removal of an inhibitory factor provided by the fibre, or by changes in the extracellular concentration of calcium (Reznik 1969).

iii. Connective Tissue Framework of Muscle

Investigations reported during the early 1980's provided evidence that the extracellular matrix (connective tissue) acts in many ways to control the development and cellular metabolism of the muscle fibre. Prior to this time, it was believed that the extracellular matrix was not metabolically active and merely provided mechanical support to the muscle fibre (Hay 1981, Heathcote & Grant 1981, Trelstad 1984).

The extracellular matrix of skeletal muscle is organized into three separate but interconnected sheaths. The epimysium is the outermost sheath that surrounds the entire muscle. Extending inward are smaller bundles of coarse collagen fibres which subdivide the muscle into compartments; these are termed the perimysium. Nerve and vascular branches are found in the perimysium. Finally, each fibre within the perimysiun is invested with a sheath of endomysium. The endomysial sheath is comprised of three separate fibres: collagen, reticulin, and elastin (Postlethwaite & Smith 1987). When intermingled they form a fibrous matrix sheath. The space between the fibres is filled with an amorphous ground substance comprised of a mixture of acidic proteoglycans, glycoproteins (chondronectin, fibronectin, and laminin) and a few less well characterized minor components (Engle 1986). A similar sheath also encapsulates the motor nerve branches and capillaries (Hay 1981). Also contained within the matrix are the basement membrane and the plasma membrane of the muscle fibre. The basement membrane is comprised of two structures: the reticular lamina (contains fibronectin, collagen V) and the basal lamina (laminin, fibronectin, collagen IV). Lying beneath these structures is the plasma membrane which has an important transport role in muscle physiology (Singer & Nicolson 1972). Following degeneration and during the early phase of regeneration of a muscle fibre, it is the basement membrane which appears to serve as a conduit or guide for the newly forming fibre (Carpenter & Karpati 1984). If the basement membrane is ruptured as a result of the injury, cellular regeneration is not impaired; however, myotubes are not aligned and the functional capacity may be jeapordized (Bischoff 1986). The plasma membrane of the muscle is similar in structure to other plasma membranes of the body. It is a fluid mosaic membrane composed of a lipid bilayer and proteins (Carpenter & Karpati 1984). When the plasma membrane is damaged following trauma, massive influxes of extracellular calcium enter the muscle fibre. This occurs at the site of the calcium channel or of gross rupture of the membrane, and results in damage to mitrochondria and the activation of proteolytic enzymes (Wrogemann & Pena 1976).

iv. Fibroblasts

Fibroblasts are specialized stromal cells that play an important role in the repair of the perimysial and endomysial connective tissues. Fibroblasts repair connective tissue that has been torn by mechanical trauma (Cohen & Moore 1979). A number of reports have established that fibroblasts do not function autonomously, but instead respond to distinct molecular signals such as transforming growth factor, platelet-derived growth factor, fibronectin, and serum(C5)-derived chemotactic factor (Postlethwaite & Snyderman 1979). Activated fibroblasts in the damaged area proliferate and secrete tropocollagen, which is necessary for the formation of the extracellular matrix, which in turn is involved in the morphogenesis of the developing fibre (Chevallier & Kieng 1977, Engle 1986). Once inflammation has subsided and regeneration is complete, the fibroblasts continue to synthesize and maintain the extracellular matrix. Indeed, the collagen remains in a continuous state of slow remodelling and is degraded by enzymes released by the fibroblast (Cohen & Moore 1979).

In summary, for effective muscle regeneration to occur, the two surviving stumps of the injured muscle fibre that face the intervening necrotic segment must be encased by an intact sarcolemmal membrane. Myoblasts which arise from the satellite cells fuse with one another to form myotubes which elongate or grow in a lateral direction to reform the injured segment (Carpenter & Karpati 1984, Engle 1986). The preceeding events occur first at the periphery and then proceed towards the centre of the newly forming segment (Woo & Buckwalker 1977). Within the myotube, plump centrally located nuclei are surrounded by aggregates of myofilaments in a clear cytoplasm. Sarcomeres organize and continue to expand in the sarcoplasm until the myotube is completely filled to form a functional fibre again (Carpenter 1977, Engle 1986).

F. REGULATION OF PROTEIN TURNOVER IN SKELETAL MUSCLE

Proteins are the main constituents of muscle fibres and undergo continual breakdown and replacement by the synthesis of new protein (Goldberg et al. 1980, Goldberg & St. John 1976, Garlick & McNurlan 1980) a process referred to as protein turnover. Tissue protein levels are determined by both the rates of protein synthesis and protein degradation (Goldberg et al. 1980, Fulks et al. 1975). The overall balance between rates of protein degradation and synthesis determines whether the muscle is experiencing atrophy or growth. Muscle injury produces an increase in protein catabolism resulting in a decrease in protein content of the injured muscle. Following injury, this muscle protein loss and subsequent regrowth would also appear from alterations in the relative rates of protein synthesis and degradation; however, to date these changes have only been partially characterized despite their obvious importance for sports medicine and surgery (Tischler et al. 1982).

The purpose of this section is to outline factors which are known to affect muscle protein synthesis and degradation. In particular, the possible role of prostaglandins produced during the acute inflammatory response will be raised. Diverse factors which are known to affect muscle protein turnover and summarized in Table I.2. Several of these are covered in detail below.

CIRCULATING HORMONES AND OTHER FACTORS

i. PROSTAGLANDINS

Several authors have suggested that PGE_2 and $PGF_{2\alpha}$ signal changes in muscle protein turnover during several pathological and physiological states (Reeds & Palmer 1984a, Tischler et al. 1982). PGE_2 increased protein degradation (without affecting protein synthesis) (Rodemann & Goldberg 1982) whereas $PGF_{2\alpha}$ stimulated protein synthesis without affecting breakdown (Reeds et al. 1985, Rodemann & Goldberg 1982). Skeletal muscle synthesizes and releases PGE_2 and PGF_2 (Nowak et al. 1983, Rodemann & Goldberg 1982) and this process is stimulated by the addition of arachidonic acid (Rodemann & Goldberg 1982).

In addition to the *in vitro* studies cited above, the importance of prostaglandins in regulation of protein turnover *in vivo* has begun to be explored. Prostaglandin $F_{2\alpha}$ may be important in several instances of net muscle anabolism. For example, Reeds & Palmer 1985 have demonstrated that the activation of muscle protein synthesis by insulin depends on $PGF_{2\alpha}$. The catabolic effects of the synthetic glucocorticoid dexamethasone, appears to result from suppression of muscle $PGF_{2\alpha}$ synthesis and hence of protein synthesis (Reeds et al. 1983). Finally, the repletion of muscle protein following disuse atrophy appears to be $PGF_{2\alpha}$- dependant (Templeton et al. 1986). These authors reported that the administration of

Table 3.Factors reported in the literature that influence protein turnover in skeletal muscle

Factor	Protein synthesis	Protein degradation	Net effect
Insulin	increase	decrease	anabolic
Insulin-like growth factor I and II	increase	decrease	anabolic
Growth hormone	increase	no effect	anabolic
Testosterone & Estradiol	increase	decrease	anabolic
Triiodothyronine			
Physiological dose	increase	increase	no change
Hyperthyroid dose	increase	increase	catabolic
Glucagon	no effect	increase	catabolic
Glucocorticoids			
Fed state			
Low dose	decrease	no effect	catabolic
High dose	decrease	increase	catabolic
Fasted state	decrease	increase	catabolic
PGE_2	no effect	increase	catabolic
PGF_2	increase	no effect	anabolic
Muscle contraction	increase	increase	anabolic
Starvation			
short term	decrease	increase	catabolic
long term	decrease	decrease	preserve N
Calcium	no effect	increase	catabolic
Magnesium	no effect	decrease	anabolic
Leucine	increase	decrease	anabolic
alpha-ketoisocaproate	no effect	decrease	anabolic
Glucose	no effect	decrease	anabolic
Glutamine	increase?	decrease?	anabolic?
Ketone bodies	decrease?	decrease?	preserve N?

the NSAID indomethacin inhibits soleus muscle hypertrophy following recovery from disuse atrophy by inhibiting protein synthesis within the muscle.

PGE_2 has been demonstrated to activate muscle protein degradation in vivo. Strelkov et al. (1989) demonstrated that PGE_2, originating from a tumour, activated muscle protein degradation in the laboratory rat. Similarly, the catabolic effect of fever and infection on muscle would appear to be mediated by PGE_2 (Baracos et al. 1988; Tian & Baracos, (1989) reported that domestic chicks and rats treated with naproxen, an inhibitor of prostaglandin synthesis, reduced weight losses of body and muscle and significantly inhibited muscle protein wasting. Although evidence for the role of PGE_2 in regulation of muscle protein turnover in catabolic states is available, no studies have been carried out on injured muscle. The participation of PGE_2 in inflammation is well-documented (see above), and it appears likely that this factor could be responsible for regulation of catabolism of injured muscle. Furthermore, inhibitors of prostaglandin synthesis are widely used subsequent to muscle injury; these agents may have an important impact on protein turnover after injury, of prostaglandins are involved.

ii. Insulin

Manchester & Young (1960) were among the first to report that elevated levels of insulin stimulate amino acid uptake in isolated muscle. Following a meal, the release of insulin promotes a net accumulation of protein in skeletal muscle. In fasting insulin levels drop, producing a net protein loss and release of amino acids from the muscle (Rodemann & Goldberg 1982). Further studies have shown that insulin has an anabolic effect on muscle by: (1) enhancing protein synthesis by promoting the initiation of protein translation (Fulks et al. 1975), by inhibiting protein degradation (Goldberg et al. 1980, Jefferson & Rannels 1974), and by promoting amino acid transport into the muscle by the activation of a sodium-dependant transport system (Kettelhut et al. 1988). Within skeletal muscle, insulin alters the growth of lysosomes by preventing them from achieving normal size (Jefferson & Rannels 1974, Rannels et al. 1978).

iii. Growth Hormone

It is well established that impaired muscle growth occurs in hypophysectomized animals (Kostyo & Redmond 1966). This alteration in endocrine function decreases the rate of protein synthesis in skeletal muscle and alters the RNA content of the nucleus (Goldberg et al. 1980, Flaim et al. 1978). These effects can be reversed and overall muscle growth can be reestablished by the administration of pituitary growth hormone and thyroid hormone. However, the rate of protein breakdown also decreases in the hypophysectomized rat and that the administration of growth hormone fails to increase proteolysis (Flaim et al. 1978). Recently, a number of studies have shown that a decreased level of thyroid hormone produces a sparing effect in skeletal muscle following hypophysectomy (Goldberg & Goldspink 1975, Griffin & Golderg 1977). When animals were treated with either triiodothyronine or thyroxin, decreased levels in protein degradation were observed (Griffin & Goldberg 1977). Growth hormone and thyroxin are both essential for protein synthesis within the muscle.

iv. Glucocorticoids

The glucocorticoids are another group of hormones which are capable of influencing muscle mass (Florini 1987). In man, a condition known as Cushing's disease is caused by the production of excessive amounts of corticosteroids. This results in muscle weakness, atrophy, and necrosis of the muscle fibre (Adams 1974). Florini (1987) has referred to the glucocorticoids as being protein catabolic hormones. Their presence leads to a net loss of muscle proteins and the conversion of amino acids into carbohydrates. This appears to involve three mechanisms: (1) an increased release of amino acids by the muscle, (2) a decrease of DNA and protein synthesis at the translation level, and (3) a reduction of amino acid uptake by the muscle (Kostyo and Redmond 1966, Rannels et al. 1978b, McGrath 1987). Kelly & McGrath (1986) and McGrath & Goldspink (1987) reported a preference for fibre type in the catabolic activity of glucocorticoids in muscle, with fibres (extensor digitorum longus) being highly sensitive and fibres (soleus) being less sensitive. One final mechanism involving the catabolic action of cortisol reported in the work of Tan & Bonen (1985), was that increased levels of cortisol produce an antagonistic effect on the insulin receptor.

v. Anabolic steroids

Anabolic steroids are synthetic derivatives of the male hormone testosterone which is the principal androgen (Goodman & Gilamn 1985). Androgens serve different functions depending on the age of the individual. During embryonic development, their central role is in the development of the male phenotype and they serve to virilize the urogenital tract of the male embryo (Franchemont 1977). Within the male, during the early stages of puberty, the growth-promoting properties of the androgens produce an increase in height and the development of the skeletal musculature. Since androgens were reported to have a significant effect on muscle mass in hypogonadal men, it was later assumed by the athletic community, but never proven, that androgens could promote growth of muscle above normal values (Wilson & Griffin 1980). The rationale for this assumption was based on the belief that androgenic and anabolic effects were different and a large undertaking to produce a pure anabolic steroid without an androgenic effect was undertaken. However, Wilson and Griffin (1980), reported that anabolic and androgenic responses do not result from different actions of the same hormone but represent the actions in different tissues: muscle which responds androgenicly also possesses the identical receptor which mediates the action of the hormone in other target tissues (Wilson & Griffin 1980). The use of anabolic steroids in sports was first reported in the mid-1950's (Welsh & Sheppard 1986). Body builders and weight lifters were the first to use these drugs in an attempt to improve athletic performance. Today the use of anabolic steroids is wide spread and is found at all levels of competitive sport. The reviews of three separate groups of investigators (Ryan 1981, Wilson & Griffin 1980) scrutinized a total of 25 papers on the effects of androgens and anabolic steroids on athletic performance. Their final conclusion was that the use of these agents does not improve athletic performance even when extremely large doses are taken (Ryan 1981, Wilson 1988).

vi. Growth factors in skeletal muscle

Growth factors are substances which provide the biological signals involved in the regulation of cell growth and differentiation during both pre- and post-injury events. Growth factor substances are hormone related; however, in contrast to the classical hormones, growth factors are not commonly synthesized by the endocrine glands but by cells frequently located near the injury site (Pimentel 1987, Alderman et al. 1985).

Insulin-like growth factors, (IGF) also termed somatomedins, are members of a family of peptide hormones that mediate many of the growth promoting actions of growth hormone (Phillips et al. 1980). The physiological profile of IGF-1 is different from that of IGF-II. Studies have shown that IGF-I is more mitogenic and growth hormone dependant than IGF-II. IGF-II, however, is more insulin-like in its actions and is found in greater concentration in the blood (Russel & Van Wyk 1984). When IGFs are coupled with growth hormone in skeletal muscle, anabolic actions occur: there is an increase in whole body muscle mass reported by Schoelne & Zapf (1982). In the isolated muscle preparation, the soleus muscle, which has receptors for IGF-I, responds to this hormone by exhibiting an increase in amino acid and glucose uptake (Poggi et al. 1979).

vii. Fibroblast Growth Factor

Fibroblast growth factor (FGF) is another polypeptide growth factor that stimulates fibroblasts, as well as a variety of neuroectodermal and mesodermal cell types (Florini 1987). In recent studies Bischoff (1986a) found that FGF is highly mitogenic for satellite cells in the rat. Others (Linkhart & Clegg 1978, 1981) have found FGF to have a blocking effect on myoblast differentiation. This is dependant not on the cell reentering the cell cycle, but on protein synthesis (Spizz & Roman1986). Finally, the actions of FGF in inhibiting muscle growth are not related to its myogenic activity. However, Lathrop & Thomas (1985) reported that the dividing cell is inhibited in the G_0 state shortly after it enters into G_1. FGF causes the cell to remain in this position until differentiation is no longer possible.

Finally, the mechanism by which growth factors may influence cell proliferation and DNA synthesis is not fully understood. However, growth factors are reported to be regulatory substances which produce the biological signals involved in the differentiation and growth of the cell throughout its life. Some growth factors have the capacity to act in a universal manner thereby initiating a wide spectrum of activies for various types of tissues and cells, while others have selective functions for a particular cell type (Pimental 1987).

Summary

The effects of factors cited above are important in the regulation of protein turnover in skeletal muscle. It is now recognized that protein metabolism is under strict endocrine control (Florini 1987). Some factors, such as growth hormone, thyroid hormone, and anabolic steroids all have a positive effect on the muscle and promote protein synthesis. Glucocorticoids have a catabolic effect on the skeletal muscle,and also have a coordinated effect with insulin to control protein turnover. Much remains to be learned. The influence and relative importance of each factor in muscle wasting and repair following injury must ultimately be characterized.

G. TREATMENT OF SPORTS INJURIES

In this section, the topic of therapeutic ultrasound (TU) and non-steroidal anti-inflammatory drugs (NSAID) will be reviewed. These two common forms of treatment of sports injuries are topics which I have chosen to investigate for my study.

OVERVIEW

In today's athletic therapy and sports medicine, heat, cold, electrical modalities, and a number of pharmacological agents are being used to enhance the healing process and aid in the rehabilitation of the injured athlete (Reilly 1981). The application of cold during the early phase of injury is reported to produce anesthesia and slow the inflammatory process (Knight 1986). Heat has the capacity to increase circulation and provide relaxation, thereby enhancing the athlete's mental state of well being (Arnheim 1989). Short wave, microwave, and ultrasound are described as penetrating heat therapies and bring about an increase in temperature deep within the tissue (Litch 1965). Associated with the increase in tissue temperature is a corresponding increase in blood flow to the injury site which in turn accelerates the metabolic process and stimulates phagocytosis, enhancing the healing process (Arnheim 1989). These modalities are reported to reduce some of the mediators of inflammation (Fyfe & Chahl 1984, Dyson & Suckley 1978), but there is little scientific evidence that they will shorten the healing time. NSAID are useful in the treatment of sport injuries. However, the best timing for the administration of these drugs following injury is still not known (Almekindes 1986).

i. Ultrasound

In the area of sports medicine, after hot packs, the application of therapeutic ultrasound is probably the most frequently used modality for the treatment of muscle injury (Gould 1985). One reason for the popularity of ultrasound in the treatment of muscle injury is the depth of penetration and uniform heating to underlying tissue provided (Griffin 1966). In sports injury, the depth and magnitude of damage to the muscle will vary depending on the nature of the injury (Reilly 1981). Therefore, if rehabilitation is to be achieved, the modality must have sufficient penetrating quality to reach the injury site and produce a therapeutic effect. Griffin 1966) reports ultrasound provides the greatest energy per unit time when compared to short wave diathermy or moist heat. In addition, as the energy per unit in time is increased (penetration) a more unified rate of rise of tissue temperature results. This enhances the healing of the injury (Arnheim 1989).

The heating effect caused by the ultrasound is reported to cause an increase in blood flow to the injury site. The dilated blood vessels become more permeable to exudate in the extracellular space, thus allowing edema fluid to move from the injury site into the vessel (Dyson & Suckley 1978). The non-thermal effect of ultrasound is a mechanical action producing a shear force which is exerted on the cell membrane. This force is probably responsible for the increased cell membrane permeability and enhanced protein production (Harvey 1985).

A number of studies have suggested that ultrasound is capable of altering inflammation and enhancing tissue repair following soft tissue injury (Hashishe et al. 1986, Fyfe & Chahl 1985, Goddard et al. 1983). Fyfe & Chahl (1985) reported that ultrasound induces mast cell degranulation. The degranulation releases histamine which produces vasodilation and increased permeability of small blood vessels. However, the amount of histamine produced has a profound effect on healing and an over-production will impair the regeneration process (Dabrowski et al. 1975, Soeki et al. 1975). One study similar to those already cited found that ultrasound produced a reduction in inflammatory edema; however, the authors attribute the result to a strong placebo effect, possibly through massage by the head of the device (Hashishe et al. 1986). Goddard et al. (1983) implanted irritating sponges in the hind limbs of rats to examine the acute inflammatory response and found no evidence of any anti-inflammatory activity by ultrasound. These results are conflicting and the variation in findings may be attributed to the dosage that was delivered and to the duration and frequency of the application. Fyfe & Chahl (1985) reports that the effect of a single ultrasound treatment can be shown to continue for several days and that the character of the response may change during this time.

Several investigators have reported that ultrasound enhances tissue healing. Dyson & Suckley (1978) reports that low dosages of pulsed ultrasound increases tissue growth and regeneration in the injured rabbit's ear. An increase in protein synthesis by fibroblast in vitro after ultrasound treatment, was also reported (Harvey 1985). It has been shown that ultrasound encourages the formation of granulation tissue in the clinical treatment of various ulcers (Dyson & Suckley 1978). One study investigated the effects of ultrasound following acute blunt trauma. Rats were subjected to a single blow to the hind limb and then treated with ultrasound (Stratton et al. 1984). Following the treatment regime the animals were sacrificed and the muscle was examined under the light microscope. The investigator used muscle nuclei as an indicator of regeneration and reported that 1.5 watts per square centimeter dosage was highly significant as compared with a lower 0.5 watts per square centimeter dosage in promoting muscle regeneration (Stratton et al. 1984).

Therapeutic ultrasound has enjoyed a clinical notoriety during the past four decades. In the area of sports medicine ultrasound is popular for the treatment of muscle injury when deep heat is required (Griffin 1966, Stratton et al. 1984). In addition, it is also reported to alter the inflammatory response; however, there is little agreement as to the exact mechanism of action (Fyfe & Bullock 1985). Fyfe & Chahl (1984) reported that ultrasound stimulates the mast cell to release histamine, a mediator of inflammation, but did not report on the final outcome. Others (Hashishe et al. 1986, Goddard et al. 1983) have investigated the final outcome regarding inflammation and provide conflicting results. One explanation for such variation in results may relate to the duration of application and the intensity of the ultrasound. Fyfe & Bullock (1985) report that in North America the biological effects of ultrasound are determined by their thermal characteristics. An intensity of 1.5 watts per square centimeter is employed (or as high as the patient can tolerate so as to produce a deep heating effect) (Stratton et al. 1984). In Europe and Australia it is general practice to use ultrasound at a much lower frequency (0.3 to 0.75 watts) in an attempt to achieve a mechanical effect which in turn will stimulate or massage the injured tissue and enhance healing (Fyfe & Bullock 1985).

Ultrasound continues to be the most frequently used modality treating soft tissue injury. Despite the frequent clinical use and extensive investigation, many of the physiological effects of this modality are still not well understood (Kramer 1985). It is still not clear, as to the selection and use of all the factors comprising the current dosage of ultrasound when dealing with soft tissue injury (Fyfe & Bullock 1985). Finally, much of the information related to the effectiveness of ultrasound is based on macroscopic observations, and there is a general lack of research on the effects of ultrasound on traumatized tissue (Stratton et al. 1984).

ii. Non-steroidal anti-inflammatory drugs in sport

In the field of sports medicine, the administration of nonsteroidal anti-inflammatory drugs (NSAID) for the control of inflammation and relief of pain is a common form of treatment. Drawing from numerous reports (Salminen & Kihlstrom 1987, Weiler et al. 1987), it is now well established that despite differences with respect to potency, specificity, and mechanism of action, the majority of these drugs act by inhibiting cyclo-oxygenase activity (Ferreira 1972, Huskisson 1977, Kulmacz & Lands 1985). Aspirin, the most common, functions because it acetylates and irreversibly inactivates cyclo-oxygenase (Roth & Stanford 1975). Indomethacin, a more potent compound, appears to inhibit cyclo-oxygenase activity by binding in a stereospecific fashion to one of the subunits of the enzyme (Kulmacz & Lands 1985). Agents such as acetaminophenol and phenylbutazone are only effective when steady-state concentrations of lipid peroxides are reduced (Haennel & Lands 1982). These results highlight differences in metabolism and bioavailability and may explain the differences in toxicity and therapeutic efficacy reported among commonly used nonsteroidal anti-inflammatory drugs (Gallin et al. 1986). Associated with these compounds are unwanted side effects such as headache, nausea, and depression, all of which may affect athletic performance.

Recently, a number of reports have shown that the inhibition of cyclo-oxygenase activity is only one of the anti-inflammatory actions of these aspirin-like drugs. The activities of neutrophils during chemotaxis, phagocytosis, degranulation, and the release of their reactive oxygen metabolites can also be inhibited with non-sterodial anti-inflammatory drugs (Turner & Semble 1984). In a report by Salminen & Kilstrom (1987), the drug indomethacin had a protective effect on skeletal muscle following exercise. Indomethacin was administered to rats during and after exercise bouts. The results following exercise showed a significant reduction in ultrastructural damage in drug treated animals, and the enzymatic indicators of muscle necrosis (β-glucuronidase and glucose-6-phosphate dehydrogenase) were also reduced significantly in animals that received the drug. Non-steroidal anti-inflammatory agents are commonly used in sports, particularly for soft tissue injuries encountered in play. The pharmacology of these drugs is becoming well known; clinically, they are prescribed to control inflammation and reduce pain. In athletics, under nonmedical or poorly supervised conditions, the drugs are frequently used to mask pain, eliminate disability and permit continued athletic performance in spite of further injury. Team physicians and therapists must perceive such patterns and be alert to any associated drug abuse signs and symptoms (Scott 1986).

H. HYPOTHESIS AND EXPERIMENTAL PROPOSAL

From the foregoing, the following hypotheses and objectives may be stated:

Hypothesis #1: The protein mass of muscle will be modified during the course of inflammation and repair following muscle injury.

Hypothesis #2: PGE_2 is a mediator of the local inflammatory response and is likely to participate in the modification of protein mass seen in injury and/or repair of the muscle.

Hypothesis #3: The use of ultrasound or a non-steroidal anti-inflammatory drug is likely to directly alter the expression of PGE_2 and the actions of this mediator on protein repletion following injury.

The major objectives of this study will be to:

1) develop an experimental model of acute trauma to muscle tissue, such that the degeneration and regenerative responses can be studied in a controlled fashion.

2) evaluate the therapeutic effects of ultrasound and the non-steroidal anti-inflammatory drug naproxen, using this model to deliver trauma.

REFERENCES

1. Adams, S., Adrian, M. and Bayless M. 1987. Catastrophic injuries in sports. Benchmark Press, Inc., Indiannapolis, Ind. 1-9.

2. Alderman, E.M., Lobb, R., Fett, J. 1985. Isolation of tumor secreted products from human carcinomia cells. Proc. Natl. Acad. Sci. U.S.A., 82:469-475.

3. Ali, M. 1979. Myotube formation in skeletal muscle regeneration. J. Anat. 128:553-562.

4. Allbrook, D., Baker, W. 1966. Muscle regeneration in experimental animals and in man. J. Bone Joint Surg. 48B:153-169.

5. Allbrook, D., Han M. 1971. Population of muscle satellite cells in relation to age and metabolic activity. Pathology. 3:233-243

6. Almekindes, L., Jerome, M. and Gilbert, A. 1986. Healing of experimental muscle strains and the effects of non-steroidal antiinflammatory drugs. Amer. J. Sports Med. 14, 4:303-308.

7. Armstrong R.B. 1986. Muscle damage and endurance events. Sports Med. 3:370-381.

8. Armstrong, R., Ogilvie R. 1983. Eccentric exercise-induced injury to the rat skeletal muscle. J. Applied Physiol. 54:80-93.

9. Arnheim, D. 1989. Modern Principles of Athletic Training, Time Mirror/Mosby Publishing Co. St. Louis. 339-366.

10. Baracos, V., Rodemann, H.P., Dinarello, C., Goldberg, A.L. 1983. Stimulation of muscle protein degradation and prostaglandin E2 release by leukocytic pyrogen (interleukin-1). N. Eng. J. Med. 308:553-555.

11. Basson M.D., Carlson. 1980. Myotoxicity of single and repeated injections of mepiyacaine (carbocaine) in the rat. Anesth. Analg. 59:275-282.

12. Beaver, M. 1978. Histamine: Its role in physiological and pathological processes (monograph). Allergy. 13:1-20.

13. Benoit P.W., Belt W.D., 1970. Destruction and regeneration of skeletal muscle after treatment with a local anaesthetic, bupivacaine (marcaine). J. Anat. 107:547-556.

14. Bischoff, R. 1975. Regeneration of a single skeletal muscle fibre in vitro. Anat. Rec. 182. 215-236.

15. Bischoff R. 1986a. Proliferation of muscle satellite cells on intact myofibre in culture. Dev. Biol. 115. 129-139.

16. Bischoff R. 1986b. A satellite cell mitogen from crushed adult muscle. Dev. Biol. 115. 140-147.

17. Bosson, M.D., Carlson B. 1980. Myotonicity of single and repeated injection of meperacaine (carbocaine) in the rat. Anest. Anat. 59:275-282.

18. Buse, M., Reid, S. 1975. Leucine, a possible regulation of protein turnover in muscle. J. Clin. Invest. 56:1250-1260.

19. Carlson, B. 1976. A quantitative study of muscle fibre survival and regeneration in normal, pre-denervated and marcain-treated free muscle grafts in the cat. Exp. Neurol. 52:421-432.

20. Carpenter, S., Karpati, G. 1984. Pathology of Skeletal Muscle. Churchill Livingston. 170-176.

21. Chevalier, A., Kieng, M. 1977. Limb-somite relationship origin of the limb musculature. J. Embryol. Exp. Morpho. 150:170-186.

22. Cohen, I., Moore, C. 1979. Onset and localization of collagen synthesis during wound healing in the rat. Proc. Soc. Exp. Bio. Med. 160:458-462.

23. Dabrowski, R., Maslinski, C. and Olczak, A. 1975. The role of histamine in wound healing. Agent and Action. 7:219-224.

24. Davies, P., Bailey, P., Goldenberg, M. 1984. The role of arachidonic acid oxygenation products in pain and inflammation. Annu. Rev. Immunol. 2:335-337.

25. Dolwick, M.F., Bush, F.M., Seibel, H.R. 1977. Regeneration of masseter muscle following lidocaine-induced degeneration: a histochemical study. Acta Anat. 98:325-333.

26. Dyson, M., Suckley, J. 1978. Stimulation of tissue repair by ultrasound. Physiotherapy. 64(4):105-108.

27. Ferreira, S. 1971. Prostaglandins, aspirin like drugs and analgesia Nature, 240:200-203.

28. Flaim, D., Li, J.B., Jefferson, L. 1978. Effects of thyroxine on protein turnover in rat skeletal muscle. Am. J. Physiol. 235:E231-E237.

28. Florini, J. 1987. Hormonal control of muscle growth. Muscle & Nerve. 10:577-598.

29. Foster A.H., Carlson B.M. 1980. Myotoxicity of local anaesthetics and regeneration of the damaged muscle fibers. Anesth. Analg. 59:727-736.

30. Franchemont, P. 1977. Pituitary gonadotropin. Clin. Endocrinal. Metab. 6:101-110.

31. Friden, J.U.S., Ostrom, M. 1983. Myofibrillar damage following intense eccentric exercise. Int. J. Sport Med. 4:170-176.

32. Fulks, R., Li, J.B., Goldberg, A.L. 1975. Effects of insulin, glucose and amino acid on protein turnover in rat diaphragm. J. Biol.Chem. 250:290-298.

33. Fyfe, M., Bullock, M. 1985. Therapeutic ultrasound: Some historical background and development in knowledge of its effects on healing. Aust. J.of Physiother. 31(6):222-224.

34. Fyfe, M., Chahl, L. 1984. Mast cell degranulation and increased vascular permeability induced by therapeutic ultrasound. Br. J. Exp. Path. 65:671-676.

35. Fyfe, M., Chahl, L. 1985. The effects of single or repeated application of therapeutic ultrasound on plasma extravasation Ultrasound in Med. and Biol. 11(2):273-283.

36. Gallin, J., Goldstein, I., Snyderman, R. 1986. Inflammation, Basic Principles and Clinical Correlation. Raven Press. Toronto.129-168.

37. Garlick, P. McNurlan, Fern. 1980. A stimulation of protein synthesis and breakdown by vaccination. Brit. Med. J. 281:263-265.

38. Garrett, W., Safran, M., Seaber, A. 1987. Biomechanical comparison of stimulated and nonstimulated skeletal muscle pulled to failure. Am. J. Sport Med. 155(5):448-454.

39. Gibson, M.C., Schultz, E. 1983. Age-related differences in absolute numbers of skeletal muscle satellite cells. Muscle and Nerve. 574-580.

40. Giddings, C.J., Neaves C.B., Gonyea, W.J. 1985. Muscle fiber necrosis and regeneration induced by prolonged weight lifting exercise in the cat. Anat. Rec. 211:133-141.

41. Goddard, D., Revell, P., Carson, J. 1983. Ultrasound has no anti-inflammatory effect. Ann. Rheu. Dis. 42:582-584.

42. Goldberg, A.L., Tischler, M.E., DeMartino, G. 1980. Hormonal regulation of protein degradation and synthesis in skeletal muscle Fed. Proc. 39:31.

43. Goldberg, F., St.John, A. 1976. Intracellular protein degradation in mammalian and bacterial cells. Ann. Rev. Biochem. 45:747-803.

44. Goldberg, A.L., Griffin, G. 1977. Regulation of protein turnover in normal and dystrophic muscles, in Rowlan LP (ed) Pathogenesis of Human Muscular Dystrophies. Amsterdam Exerpta Medica. 376-385.

45. Goldberg, A.L., Goldspink D. 1975. Influence of food deprivation and adrenal steroids on DNA synthesis in various mammalian tissue Am. J. Physiol. 228:310.

46. Goldstein, I., Malmsten, C., Samuelsson, B. 1977. Prostaglandin, thromboxanes, and polymorphonuclear leukocytes. Mediation and modulation of inflammation. Inflammation. 2:309-317.

47. Goodman-Gilman A., Goodman L.S., Rall T., Murad F. 1985. The Pharmacological Basis of Therapeutics. MacMillan Publishing Co. New York. 604-715.

48. Griffin, G., Goldberg, A.L. 1977. Hormonal control of protein synthesis and degradation in rat skeletal muscle. J. Physiol. London. 270:51-52.

49. Griffin, J., 1966. Physiological effects of ultrasound as it is used clinically. J. Amer. Physiol. Ther. Assoc. Vol 46(1):1-26.

50. Gutmann, E., Carlson, B.M. 1978. The regeneration of a hormone-sensitive muscle (levator ani) in the rat. Exp. Neur. 58:535-548.

51. Haennel, A. Lands, W. 1982. Modification of anti-inflammatory drugs effects by ambient lipid peroxides. Biochem. Pharmacol. 31:3307-3311.

52. Harvey, J. 1985. Rehabilitation of the injured athlete. Clinics in Sports Medicine. 4(3):431-439.

53. Hashishe, I., Harvey, W., Harris, M. 1986. Anti-inflammatory effect of ultrasound therapy: evidence for a major placebo effect. Brit. J. Rheum. 25:77-81.

54. Hay, E. 1981. Cell Biology of Extracellular Matrix. New York, Plenum Press.

55. Heathcote J., Grant, M. 1981. The molecular organization of basement membrane. Int. Rev. Connect. Tissue Res. 9:191-264.

56. Hickson R.C., Galassi T.M., Daniels D.G. 1983. Skeletal muscle cytosol methyltrienalone receptor binding and serum androgens. J. Steroid Biochem. Vol (6):1705-1712.

57. Hickson R.C. and T.G. Hurowski. 1986. Anabolic steroid and training. Clinics in Sports Med. 5:3 July, 461-471.

58. Holtzer, H., Lash, J., Swift, H. 1970. Regeneration of mature skeletal muscle. Anat. Rec. 128:679-697.

59. Hoppeler H. 1986. Exercise-induced ultrastructural changes in skeletal muscle. Int. J. Sport Med. 7:187-204.

60. Hudgson, P., Field, E. 1973. Regeneration of muscle. New York Academic Press Vol 2. 311-363.

61. Huskisson, E. 1977. Anti-inflammatory drugs. Semin. Arth. Rheum. 7:1-20.

62. Jackson, D. 1978. Shinsplints: an up-date. Phys. Sportsmed. 6:10.

63. Jarvinen, M., Sorvari, T. 1975. Healing of crush injury in the rat striated muscle. Acta. Pathol. Microbiol. Scand. 83:259-265.

64. Jefferson, L.S., Rannels, D. 1974. Insulin in the regulation of protein turnover in heart and skeletal muscle. Fed. Proc. 33:1098-1104.

65. Keenan, E.J. 1988. Anabolic and Androgenic Steroids Drugs, Athletic and Physical Performance, Plenum Publishing Corp. New York, 91-102.

66. Kelly, F., McGrath, J. 1986. A morphological/biological study of the actions of corticosteroids on rat skeletal muscle. Muscle & Nerve. 9:1-10.

67. Kettelhut, I.C., Wing, S.S., Goldberg A.L. 1988. Endocrine regulation of protein breakdown in skeletal muscle. Diab. Metab. Rev. 4(8):751-772.

68. Kostyo, J., Redmond, A. 1966. Role of protein synthesis in the inhibitory action of adrenal steroids hormones on amino acid transport in muscle. Endocrinology. 79:531-540

69. Kramer, J. 1985. Effects of ultrasound intensity on sensory nerve conduction velocity. Physiother. Can. 37,(1) 6-10

70. Kulmacz, R., Lands, J. 1985. Stoichiometry and kinetics of the interactions of prostaglandin H synthesis with anti-inflammatory agents. J. Biol. Chem. 260:12572-12578.

71. Lathrop, B. Thomas, K. 1985. Control of myogenic differentiation by fibroblast growth factor is mediated by position GI phase of the cell cycle. J. Cell Biol. 101:2194-2198.

72. LeGros Clark, W.E., Blomfield, L.B. 1945. The efficiency of intramuscluar anastomoses with observation on the regeneration of devascularized muscle. J. Anat. 79:129-161.

73. Linkhart, T., Clegg, C. 1978. Proliferation and differentiation of mouse myoblast in vitro. J. Cell Biol. 79:25.

74. Linkhart, T., Clegg, C. 1981. Myogenic differentiation in permanent clonal mouse myoblast cell lines. Dev. Biol. 86:19-30.

75. Makitie J., Vetavamen H. 1977. Histochemical studies of striated muscle after temporary ischemia in the rat. Act Neuropath. 37:101-109.

76. Maltin C., Harris, J., Cullen M. 1983. Regeneration of mammalian skeletal muscle following the injection of snake-venom toxins Cell Tissue Res. 232:565-577.

77. Manchest K., Young F. 1960. The influence of the induction of alloxan-diabetes on the incorporation of amino acids into protein of rat diaphragms. Biochem. J. Vol.77, 386-99.

78. McGrath, J., Goldspink, D. 1982. Glucocorticoid action on protein synthesis and protein breakdown in isolated muscles. Biochem. J. 206:641-648.

79. Moss, F., Leblond, C. 1971. Satellite cells are a source of nuclei in muscles of growing rats. Anat. Rec. 170:421-35.

80. Nikolaou, M., Macdonald, M., Glisson, R. 1987. Biomechanical and histological evaluation of muscle after controlled strain injury. Am. J. Sport Med. 15: 9-14.

81. Nowak, J., Bohman, S., Alster, P. 1983. Biosynthesis of prostaglandins in microsomes of human skeletal muscle and kidney Prostag. Leukotrienes Med. 11:269-279.

82. Ontell, M., Kozeka, K. 1984. Organogenesis of the mouse extensor digitorum longus muscle: A quantitative study. Am. J. Anat. 171:149-161.

83. Ownby, C. Odell G., 1983 Pathogenesis of skeletal muscle induced by tarantula venom. Exp. Mol. Pathol. 38:283-296.

84. Partridge T.D., Slapen, J. 1977. A host contribution to the regeneration of muscle grafts. J. Neurol. Sci. 33:425-435.

85. Phillips, L., Vassilopoulou-Sellin, R. 1980. Somatomedins. New Eng. J. Med. 302:371-377.

86. Pimentel, E. 1987. Hormones, Growth Factors and Oncogenes. C.R.C. Press Inc. Boca Raton, Florida. 1-23.

87. Poggi, C., le Marchand-Brustel, Y. 1979. Effects and binding of insulin-like growth factor I in the isolated soleus muscle. Endocrinology. 105:723-770.

88. Posthethwaite, A., Smith, G. 1987. Modulation of fibroblast function *in vitro*. J. Immunol. 132:1370-2477.

89. Posthethwaite, A., Snyderman, R. 1967. The chemotactic attraction of human fibroblasts to a lymphocyte-derived factor. J. Exp. Med. 144:1188-1203.

90. Posthethwaite, A., Snyderman, R. 1979. Generation of fibroblast chemotactic factor in serum by activation of complements. J. Clin. Invest. 64:1379-1885.

91. Queiroz L.S., Santo Neto H., Rodrigues-Simioni L. 1984. Muscle necrosis and regeneration after envenomation by Bothrops jararacussu snake venom. Toxicol. 22:339-346.

92. Rannels, D.E., Kao, R., Morgan H.E.1975. Effects of insulin on protein turnover in heart muscle. J. Biol. Chem. 250:1694-1701.

93. Rannels, S., Rannels, D.E. 1978. Glucocorticoids effects on peptide-chain initiation in skeletal muscle and heart. Am. J. Physiol. 235:E134-142.

94. Rawn, D. 1989, Biochemistry, Neil Patterson Co. Burlington, North Carolina. 19-209.

95. Reeds, P.J., Glennie, R., Garlick, P. 1985. The effects of indomethacin on the stimulation of protein synthesis by insulin in young post-absorptive rats. Biochem J. 227:255-261.

96. Reeds, P.J., Palmer, R. The possible involvement of prostaglandin $F_{2\alpha}$ in the stimulation of muscle protein synthesis by insulin. Biochem. Biophys. Res. Commun. 3:1084-1094.

97. Reeds, P.J., Palmer, R. 1984. Changes in prostaglandins release associated with inhibition of muscle protein synthesis by dexamethasone. Biochem. J. 219:953-957.

98. Reilly, T. 1981. Sports, Fitness and Sports Injuries. Faber and Faber, London. 259-265.

99. Rennie, M., Hundal, H. 1986. Characteristics of glutamine carrier in skeletal muscle having important consequences for nitrogen loss in injury and infection. Lancet. N 1 1008-1011.

100. Rodemann, H.P., Goldberg A.L. 1982. Arachidonic acid, prostaglandins E_2 and $F_{2\alpha}$ influences rates of protein turnover in skeletal muscle and cardiac muscle. J. Biol. Chem. 257:1632.

101. Roth, G., Stanford, N. 1975. Acetylation of prostaglandin synthesis by aspirin. Proc. Natl. Acad. ~~Sci. USA.~~ 72:3073-3076.

102. Rubin, E., Farber, J. 1988. Pathology. J B Lippincott Co., Toronto. 1-64

103. Russell, W., Van Wyk J. 1984. Inhibition of the mitogenic effects of plasma by a monoclonal antibody to somatomedin. Proc. Natl. Acad. Sci. USA. 81:2389-2392

104. Ryan, A.J. 1981. Anabolic steroids are fools gold. Fed. Proc. 40:2682-2688.

105. Salminen, A., Kihlstrom, M. 1987. Protective effects of indomethacin against exercise-induced injury in mouse skeletal muscle fiber. Int. J. Sport Med. 8:46-49.

106. Santavista, S., Luoma, A., Arstilu, A. 1979. Morphological and biochemical changes in striated muscle after experimental tourniquet ischemia. Res. Exp. Med. 174:245-251.

107. Schmalbruch, H., Hellhammer, U. 1976. The number of satellite cells in normal human muscle. Anat. Rec. 185:279-287.

108. Schoelne, E., Zapf, J. 1982. Insulin-like growth factors-I stimulates growth in hypophysectomized rats. Nature. 296:252-253.

109. Schultz, D. Jaryszak, L., Gibson, M. 1986. Absence of exogenous satellite cell contribution to regeneration of frozen skeletal muscle. J. Muscle Res. Cell Motil. 7:361-367.

110. Scott, J. 1986. Principles of Sport Medicine. William and Wilkins, Baltimore, London. 107-111.

111. Shafiq, S., Gorycki, M. 1966. Regeneration in skeletal muscle of mouse. J. Pathol. Bacteriol. 90:123-127.

112. Singer S., Nicolson, G. 1972. The fluid-mosiac model of the structure of cell membranes. Science. 175:720-731.

113. Snow, M. 1977. Myogenic cell formation in regenerating rat skeletal muscle II, an autoradiographic study. Anat. Rec. 188:201-218.

114. Soeki, K., Yokoyam, J., Wake, K. 1975. Inhibition of granulation tissue growth by histamine. J. Pharm. Expert. Therapies. 193: 910-917.

115. Spizz, G., Roman, D. 1986. Serum and fibroblast growth factor inhibit myogenic differentiation nonfusing muscle cell line. J. Biol. Chem. 259:2337-2345.

116. Stratton, S., Hickmann,R., Rulon, F. 1984. Therapeutic ultrasound: Its effects on the integrity of a non-penetrating wound. J. Orthop. Sport Physio. Ther. 5:278-281.

117. Studitsky A.M. 1959. The Experimental Surgery of Muscle. Moscow, Izdatel Akad. Nauk. SSSR. 338.

118. Tan, M., Bonen, A., 1985. The in vitro effects of corticosterone on insulin binding and glucose metabolism in mouse skeletal muscle Can. J. Physiol. Pharmacol. 63:1133-1138.

119. Templeton, G.H., Padalino, M., Moss, R. 1986. Influences of inactivity and indomethacin on soleus phosphatidlethanolamine and size. Prostaglandin. 31:545-550.

120. Tischler, M. A., Desautels, M., Goldberg, A.L. 1982. Does leucine, leucyl-tRNA or some metabolite of leucine regulate protein synthesis in skeletal muscle. J. Biol. Chem. 257:1613-1621.

121. Trelstad, R. 1984. The Role of Extracellular Matrix in Development. New York, Alan R. Liss. 14-29.

122. Tu, A.T. 1983. Local tissue damaging (hemorrhage and myonecrosis) toxins from rattlesnake and other pit viper venoms. J. Toxicol. Toxin Rev. 2:205-234.

123. Turner, R., Semble, E. 1984. Effects of benoraprofen on human neutrophil function. J. Rheum. 11:265-271.

124. Vane, J.R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. Nature. 231:232-239.

125. Wadler, G.I., Hainline B. 1989. Drugs and the Athlete. F.A. Davis Co, Philadelphia, 53-66.

126. Warhol, M., Seigel, A., Evans, W. 1985. Skeletal muscle injury and repair in marathon runners after competition. Am. J. Pathol. 118:331-339.

127. Weiler, D. Albright, J. Buckwater, A. 1987. Nonsteroidal anti-inflammatory drugs, mechanism and clinical use. Elsevier. New York. 89-97.

128. Welsh, P., Sheppard, R.J. 1986. Current Therapies in Sport Medicine. Decker Inc. Toronto. 109-110.

129. Wilson, J.D., Griffin, J.G. 1980. The use and misuse of androgens. Metabolism. 29:1278-1295.

130. Wilson, J.D. 1988. Androgen abuse by athletes. Endocrine Reviews. 9(2):181-199.

131. Woo, S., Buckwalker, J. 1977. Injury and repair of musculoskelatal soft tissue. Am. Acad. Orth. Surg. 262-265.

132. Woolf, N. 1986. Cell Tissue and Disease. Bailliere and Tidall, London of Great Britian. 41-67.

133. Wragemann, K., Pena, S. 1976. Mitochondrial calcium overload a general mechanism for cell necrosis in muscle disease. Lancet. 672-678.

134. Yagielu, J.A., Benoit, 1981. Comparison of myotoxic effects of lidocaine with epinephrine in rats and humans. Anesth. Analg. 60:471-480.

CHAPTER II: ULTRASTRUCTURAL EVENTS FOLLOWING ACUTE BLUNT TRAUMA

A. Introduction

A large number of ultrastructural and biochemical studies have examined the response of skeletal muscle to naturally occurring diseases and experimental insults, such as ischemia, denervation, crushing, injection of local anaesthetics, and muscular dystrophies (Carlson 1983, Foster et al.Jarvinen 1975, Mastaglia et al. 1970, Solonen et al. 1968). However, relatively less attention has been paid to types of muscle damage which arise from sports activity or accidents; these may include overuse, eccentric muscle action, tearing of fibres due to stretching, and traumatic injury (Allbrook 1962, Almekinders et al. 1986, Jennische 1987 et al., Le Gross Clark 1946, Stratton et al. 1984). It has been established that skeletal muscle is capable of regeneration following injury, and that satellite cells are involved in the regenerative process (Carlson 1983a, Carlson 1983b, Gutmann et al. 1978, Mair et al. 1972, Schultz et al. 1986, Schultz et al. 1985). Information regarding the ultrastructural and biochemical events involved in muscle injury and repair is essential to the rational application of sports therapy. However, few studies have examined these events in an experimental setting (Almekinders et al. 1986, Armstrong 1986, Carlson 1983, Garrett et al. 1987, Jarvinen 1975a.b), and the impact of the commonly used therapies (e.g. ultrasound, ice, heat, laser, and non-steroidal anti-inflammatory drugs) on muscle injury and repair, has not been studied in detail at the cellular and biochemical levels. The purpose of our ongoing studies has been to develop and characterize an experimental model of acute traumatic injury to muscle.

Few experimental models for traumatic injury to muscle have been reported (Allbrook et al. 1966, Albrook 1962, Gutmann et al. 1978, Jarvinen 1975, Schultz et al. 1986, Stratton et al. 1984, Tischler et al 1983). An early study employed both bone fracture and soft tissue injury in the monkey, using a hammer device (Allbrook et al. 1966). Although the authors examined muscle and bone by transmission electron microscopy, the type of injury produced was complicated by the number of tissues involved, and was relatively severe. Tischler and Fagan (1983) reported the use of a reflex hammer which delivered multiple light blows to the posterior aspect of the lower hind limb of the rat over an extended period of time. Although this method induced considerable damage, as judged by histochemical and biochemical criteria, it may also not be representative of sports injuries. Another experimental approach has involved crushing of muscle with haemostatic forceps after opening the skin by incision (Allbrook et al. 1966, Gutmann et al. 1978, Le Gross Clark 1946, Schultz et al. 1985). Finally, the development of devices intended to deliver a standard crush injury have been reported (Jarvinen 1975, Stratton et al. 1984).

One prominent feature of accidental injuries is their variability. For example, the extent and nature of an impact of collision will vary with the amount of peak force (which depends on the mass of the object), the momentum of the object (which depends on its velocity), the area of impact, and the viscosity and compliance of the tissues receiving the impact forces applied. In the present study we have adapted the method of Stratton et al. 1984) to produce a controlled and reproducible trauma to muscle using a single blow, such as might occur in collision during contact sports or other accidental impact.

The present study reports the protein content and ultrastructural characteristics in the gastrocnemius muscle of the rats at 6 hours, and 1,2,3,6,14,21 and 30 days following acute blunt trauma, which has not hitherto been reported.

B. Materials and Methods

Animals

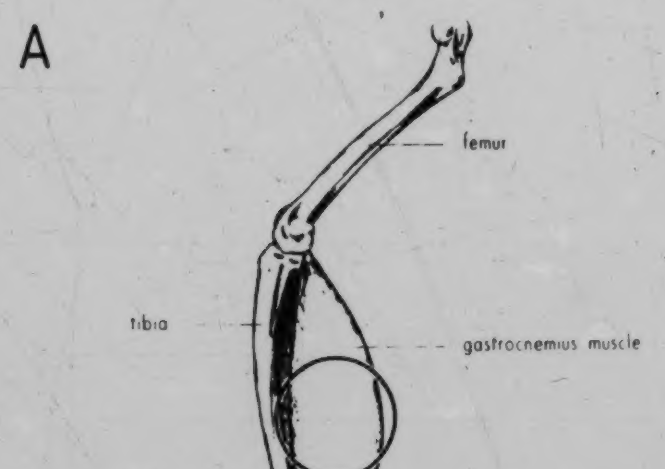
All procedures described below were carried out in conformance with the guiding principles in the care and use of animals of the American Physiological Society, and of the Canadian Council on Animal Care. Forty-eight male Sprague-Dawley rats, weighing 200-250 g at the start of the experiment, were housed individually in wire cages and provided with commercial rat chow (Wayne Lab Blox) and water ad libitum. Rats were maintained on a 12 h light: 12 h dark cycle.

Rats were subjected to a single impact trauma to the medial aspect of the gastrocnemius of the right leg, using an adaptation of the method reported by Stratton et al 1984. During this procedure rats were briefly anaesthetized with halothane. The medial side of the calf muscle was padded with a layer of gauze (0.5 cm depth) to prevent tearing of the skin. The trauma was delivered by dropping a solid aluminum cylinder with a flat impact surface (1.38 cm in diameter x 27 cm in length, weighing 700

g) once only through a distance of 125 mm, onto the padded muscle. The instantaneous force delivered by a falling object with these characteristics was calculated to equal 0.57 newton-metres/cm^2 where a newton-meter is equal to the force of an object weighing 100 g falling over a distance of 1 meter. Since the surface area of the impact device was 1.5 cm^2, the force delivered was 0.57 newton-meters/cm^2. An alternative expression of the force delivered by this device, which incorporates the velocity of object, is the momentum of impact: 1099 Kg meters/second (newton seconds). To ensure stabilization and accurate delivery, the cylinder was dropped down a tubular guide fixed to a ring stand. The limb was positioned manually with the foot stabilized at a 90 degree angle to the tibia. The location of the large belly of the gastrocnemius muscle was determined by palpation and the device placed over its broadest region. The tibia was protected from accidental fracture by carefully placing it out of the line of travel of the device; manual placement of the limb was employed primarily to avoid bone fracture. The site of skin impact was marked with indelible ink at the time of trauma to ensure subsequent localization. In Fig. 1, a scale drawing of the impact area and the sites sampled for electron microscopy is shown.

Figure 1. A. Scale drawing of impact site (circle).

B. Medial gastrocnemius muscle, sites sampled for electron microscopy (boxes).



Characterization of Trauma Response

At 6 hour, and 1,2,3,6,14,21 and 30 days after trauma, animals were sacrificed to obtain tissue samples. The medial gastrocnemius muscle was dissected intact from both the injured and uninjured limbs and subsampled for electron microscopy. At each time point 6 rats were sacrificed. Five tissue blocks were taken from each muscle (Fig. 1) from the impact site. For electron microscopy, the muscles were fixed in 2% paraformaldehyde and 4% glutaraldehyde fixative in 0.1 M cacodylate buffer at pH 7.2 (Keirnam 1981). The tissues were diced into small pieces while in fixative. After 48 h fixation at 4°C, the tissues were rinsed and post fixed in 1% buffered osmium tetroxide, dehydrated through a graded series of alcohols, and embedded in Epon and Araldite. The blocks were coded to ensure anonymity of the sample. Semi-thin sections were cut with a glass knife, stained with toluidine blue, and examined with the light microscope for the purpose of orienting tissue blocks and identifying the site of muscle trauma. Thin sections were cut with a diamond knife on the Reichert OmU2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Siemens EM 1A electron microscope.

To determine the protein content of injured muscles, separate experiments were carried out. The medial gastrocnemius muscle was dissected intact from injured and uninjured rats at 1, 3, 6, 9 and 15 days after injury in an initial experiment, and 21 and 28 d following injury in a second experiment. Muscles were solublized in 1.0 N NaOH at 50°C and protein determined by the method of Bradford (Bradford 1976).

C. Results

After trauma, rats were observed to move freely about the cage, without any noticeable limp or guarding in the injured limb. Up to 6 days after trauma, the site of impact was identified by the skin marking and the presence of a small local hematoma. Subsequently, the skin mark was used as a guide for tissue sampling. Muscle tissue was taken at 6 hours, and 1,2,3 6,14,21 and 30 days after trauma for electron microscopy.

Muscle Protein Mass

As shown in Table 1, the medial gastrocnemius muscle of young growing rats accumulated protein (+46%; $P<0.001$) over a 15 day period. Muscles from injured rats showed a marked decrease in protein content within 24 h of trauma (-35%; $P<0.01$) and a slight further decrease at day 3. After day 3, muscles accumulated protein and showed a steady rate of gain. By day 15, however, the protein content of the previously injured muscles was still only 83% of that of control muscles ($P<0.034$). Thus the injured muscles responded with a distinct catabolic phase of 3 days duration, followed by a prolonged anabolic phase. The response of individual muscles to the trauma was highly uniform, as judged by the determination of protein content.

Table 1. The effects of trauma on protein levels in medial gastrocnemius muscle

Total Protein Content (mg)

Time after Trauma (d)	0	1	3	6
Control Limb	$35.7 \pm 0.9^{*}$	34.5 ± 1.3	$43.1 \pm 2.5^{*a}$	$48.9 - 1.6^{*a}$
Trauma Limb		$30.0 \pm 1.5^{*}$	$26.2 \pm 0.5^{*b}$	$29.2 \pm 1.3^{*b}$
Trauma Control		$36.2 \pm .46^{a}$	33.8 ± 1.9^{b}	$35.8 \pm .68^{a}$

Time after Trauma (d)	9	15
Control Limb	$51.4 \pm 1.1^{*a}$	$52.1 \pm 1.0^{*a}$
Trauma Limb	36.1 ± 1.3^{b}	$43.1 \pm 1.0^{*b}$
Trauma Control	$40.2 \pm .95^{a}$	48.2 ± 1.0^{b}

* denotes different from time zero $p<0.05$. Numbers with different superscripts within the same time point are significantly different, $P<0.022$ ($n=6$). Statistical analysis was done using the unpaired students T-test. Rats were traumatized by a single blow to the medial gastrocnemius of the right leg, under general anaesthesia with halothane. Control rats were anaesthetized but not traumatized. At the time points indicated, medial gastrocnemius muscles were dissected. Total protein contents were determined after solublization in 1.0 N NaOH.

i. Ultrastructural Observations

Six Hours post-trauma

The injured muscles showed a variable pattern of tearing and disruption of normal cells and small blood vessels. Some muscle cells were completely ruptured. Other muscle cells showed varying degrees of intracellular disarray, but appeared to have an intact basement membrane (Fig. 2A). The following abnormalities were seen in all sections. Due to the local disruption of capillaries and small blood vessels, cellular constituents of the blood including erythrocytes and polymorphonuclear leukocytes escaped into the intercellular connective tissue (Fig. 2B). This was only observed at the 6 hour time point. The endomysium showed moderate edema. In one sample, a preterminal branch of an intramuscular nerve, displaying axonal damage, with formation of small vacuoles and myelin figures is shown (Fig. 2C).

One Day Post-Trauma

At 1 day post-trauma there was little evidence of residual interstitial hemorrhage. However, large numbers of mononuclear cells, which previously had not been present, were observed both in the endomysial connective tissue and focally within injured muscle cells with torn sarcolemmal tubes (Fig. 3A). Mononuclear cells were seen beneath the basement membrane of the muscle cells in focal aggregates (Fig. 3A). Activated fibroblasts were also observed in the interstitium; these possessed abundunt cisternae of rough endoplasmic reticulum (Fig. 3B).

Structural abnormalities were widespread. Injured muscle cells showed disorganization of sarcomeres and some contained large numbers of membrane-bound vacuolar structures of variable size.

Two Days Post-Trauma

At 2 days, muscle from the traumatized site contained large numbers of mononuclear cells in the endomysial connective tissue and within damaged muscle cells (Fig. 4A). Fibroblasts were also prevalent in the endomysial connective tissue. Many damaged muscle cells contained membrane-bound vacuoles of different sizes, which appeared to be derived from the sarcotubular reticulum (Fig. 4B, C); some very large clear vacuoles contained amorphous material (Fig. 4D). Large vacuoles produced compression and displacement of adjacent myofibrils. A very prominent feature of the damaged muscle cells at this time point was the presence of membraneous whorls known as myelin figures or myelin bodies. These were seen throughout the muscle fibre, particularly in a sub-sarcolemmal location (Fig. 4B).

Three Days Post-Trauma

By 3 days after trauma, mononuclear cells were infrequently observed in the endomysial connective tissue. Activated fibroblasts were still numerous at this time (Fig. 5A). For the first time, commencing focal interstitial fibrosis was observed in association with these fibroblasts.

In contrast to the previous time points, at 3 days, the traumatized muscle cells showed regenerative changes, and an increasing proportion of the muscle cells appeared normal. In muscle cells still recovering from traumatic damage, there were prominent multiple subsarcolemmal nuclei, with distinct nuclei (Fig. 5B), usually surrounded by numerous mitochondria. Satellite cells were closely applied to the muscle cells (Fig. 5B), and some were located beneath the basal lamina. A few damaged muscle fibres showed focal streaming of Z-bands (Fig. 5B).

Six Days post-trauma

At 6 days, muscle at the site of trauma appeared to have regenerated to a significant extent, so that fewer than 20% of the sections examined showed identifiable abnormalities. In these areas, numerous fibroblasts were seen lying within the endomysial connective tissue, and focal interstitial fibrosis was observed (Fig. 6A). The fibroblasts contained abundant rough endoplasmic reticulum, indicating that they were actively synthesizing protein. Satellite cells continued to be a prominent feature at this time point. Satellite cells continued to be a prominent feature at this time point. Regenerating muscle fibres displayed multiple subsarcolemmal or central nuclei with prominent nucleoli (Fig. 6B). Numerous irregularly arranged sarcomeres, composed of thick and thin myofilaments and Z-bands were identified (Fig. 6B).

14,21 and 30 days post - trauma

At these three final time points, no ultrastructural abnormalities were identified in muscle cells. Residual focal areas of fibrosis may have been present, but were not represented in the material sampled.

Figure 2. Transmission electron micrograph of gastrocnemius muscle from the injured site, 6 hours post-trauma.

A. Transverse tear (T) of a muscle cell (M), showing hypercontracted myofibrils; the basal lamina (Bm) of the cell appears intact. (X 2970).

71A

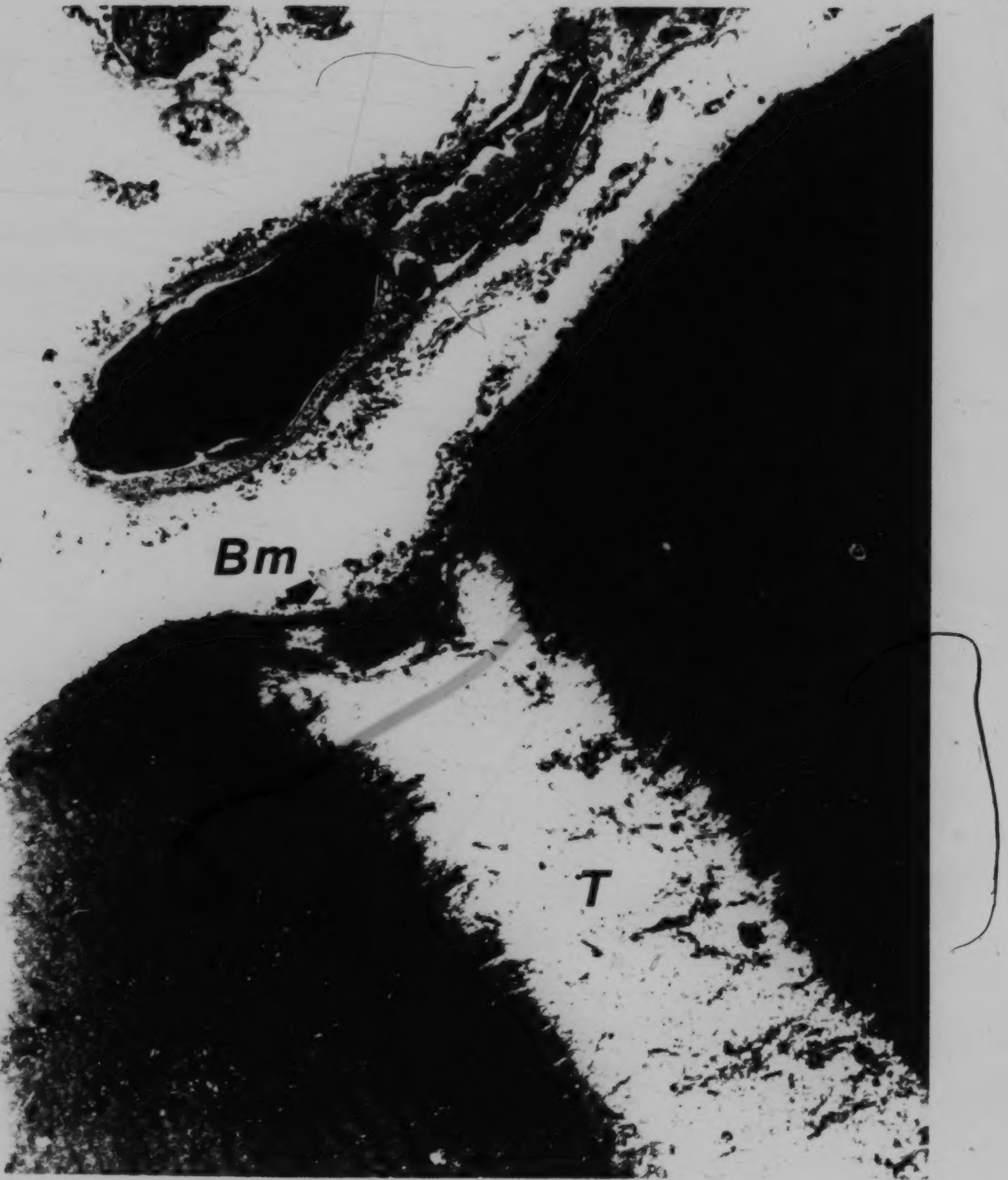


Figure 2. Transmission electron micrograph of gastrocnemius muscle from the injured site, 6 hours post-trauma.

B. Two adjacent muscle cells showing transverse tearing (M1) and extensive fragmentation (M2) of myofibrils. Polymorphonuclear leukocytes (PMn) which have escaped from ruptured capillaries are seen in the intercellular connective tissue (X 2970).

72A

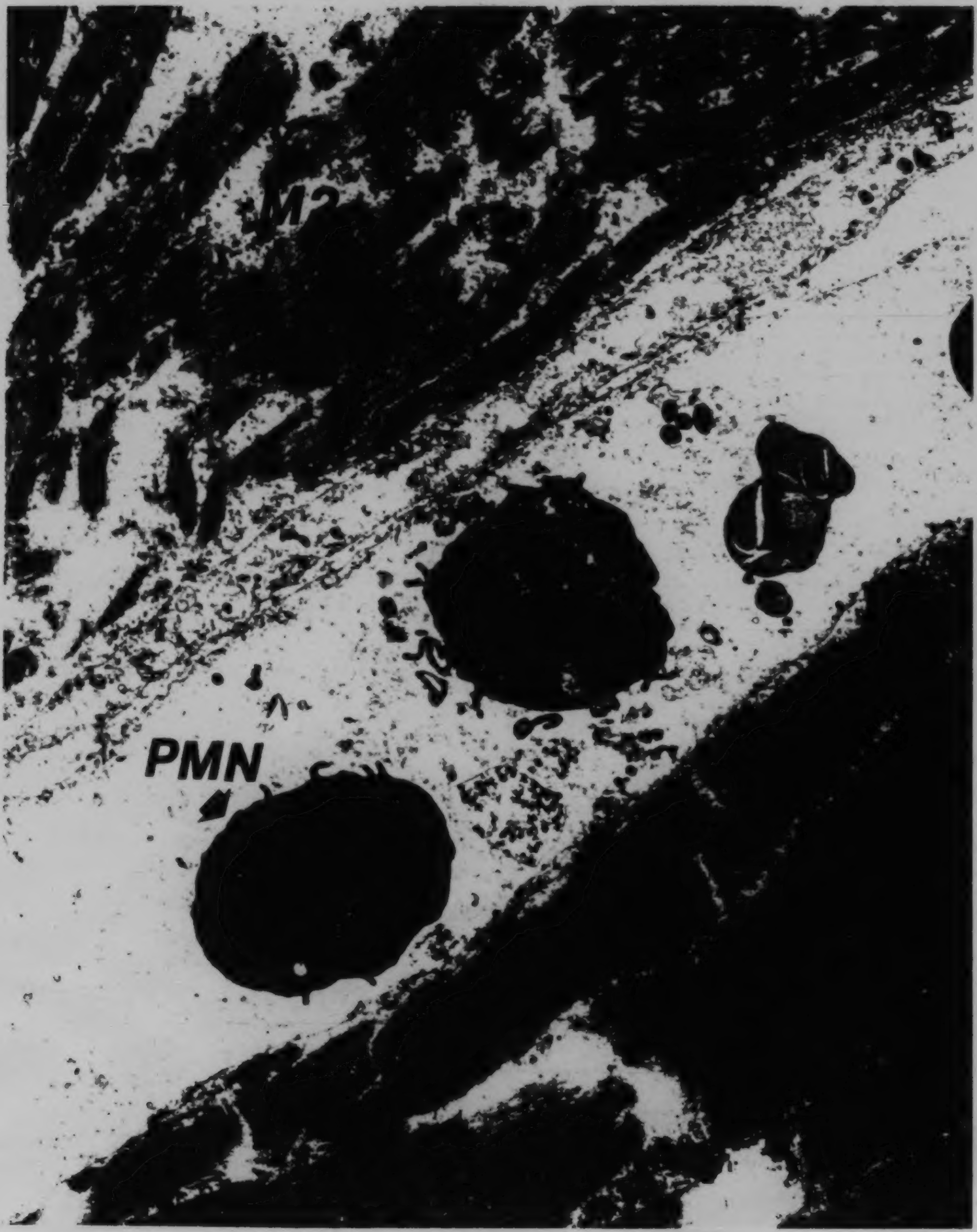


Figure 2. Transmission electron micrograph of gastrocnemius muscle from the injured site, 6 hours post-trauma.

C. Preterminal branch of an intramuscular nerve (n) between two adjacent muscle cells (M). Axonal damage, including myelin figures (W) and vacuoles (V) is observed (X2970).

73 A'

Figure 3. Transmission electron micrograph of gastrocnemius muscle from the injured site, 1 day post-trauma.

A. Central degeneration within a muscle cell (M), with the presence of mononuclear cells (P) and vacuoles (V) containing amorphous material. The endomysial connective tissue contains fine collagen fibrils (C) (X 5940).

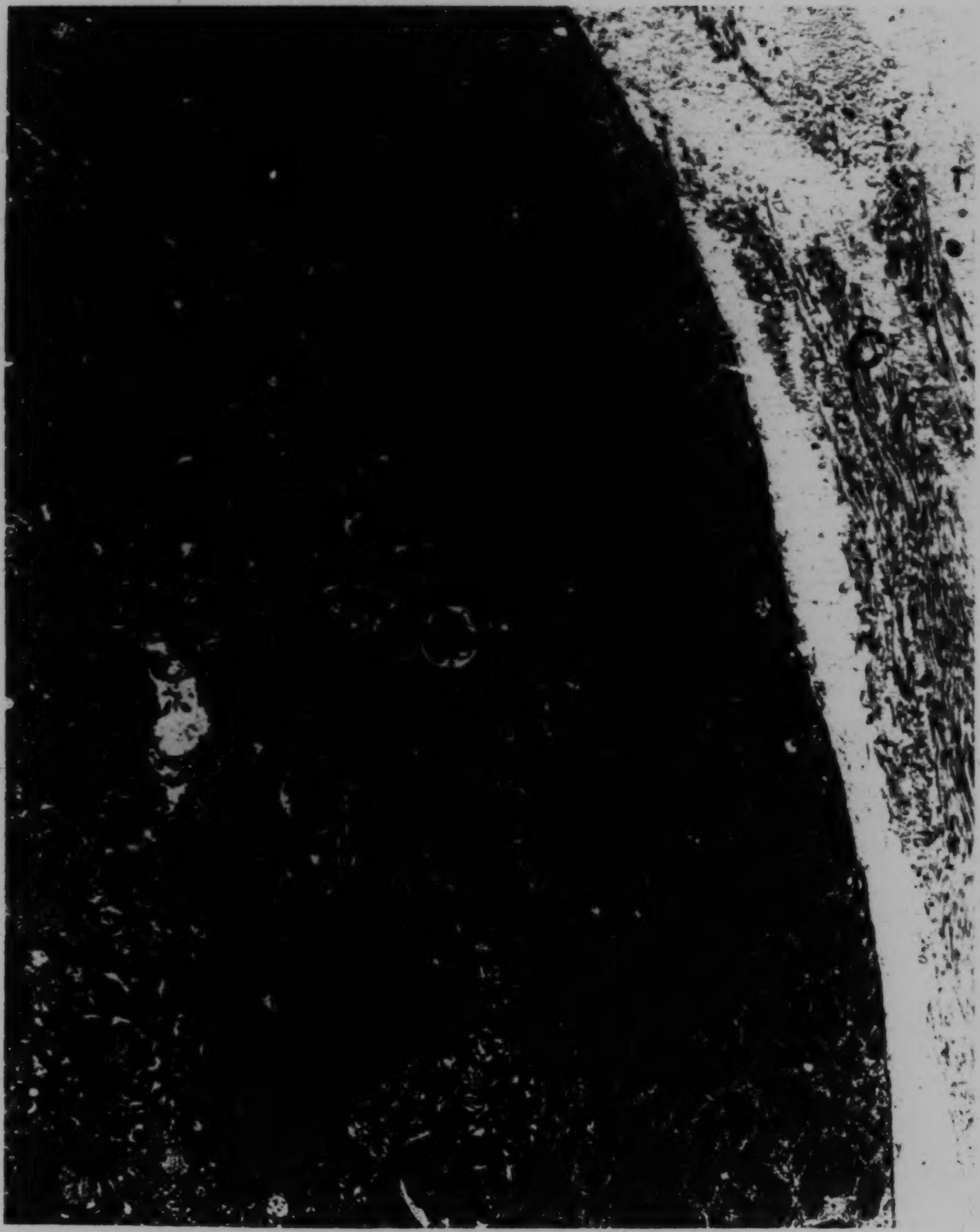

T
C

Figure 4. Transmission electron micrograph of gastrocnemius muscle from the injured site, 2 days post-trauma.

A. Section of muscle cell (m)showing myofibrils which lack organization. Membraneous whorls (W) and vacuoles (V) are also present. The endomysial connective tissue contains mononuclear cells (P) with intracytoplasmic vacuoles and numerous pseudopodia (S)(X 1815).

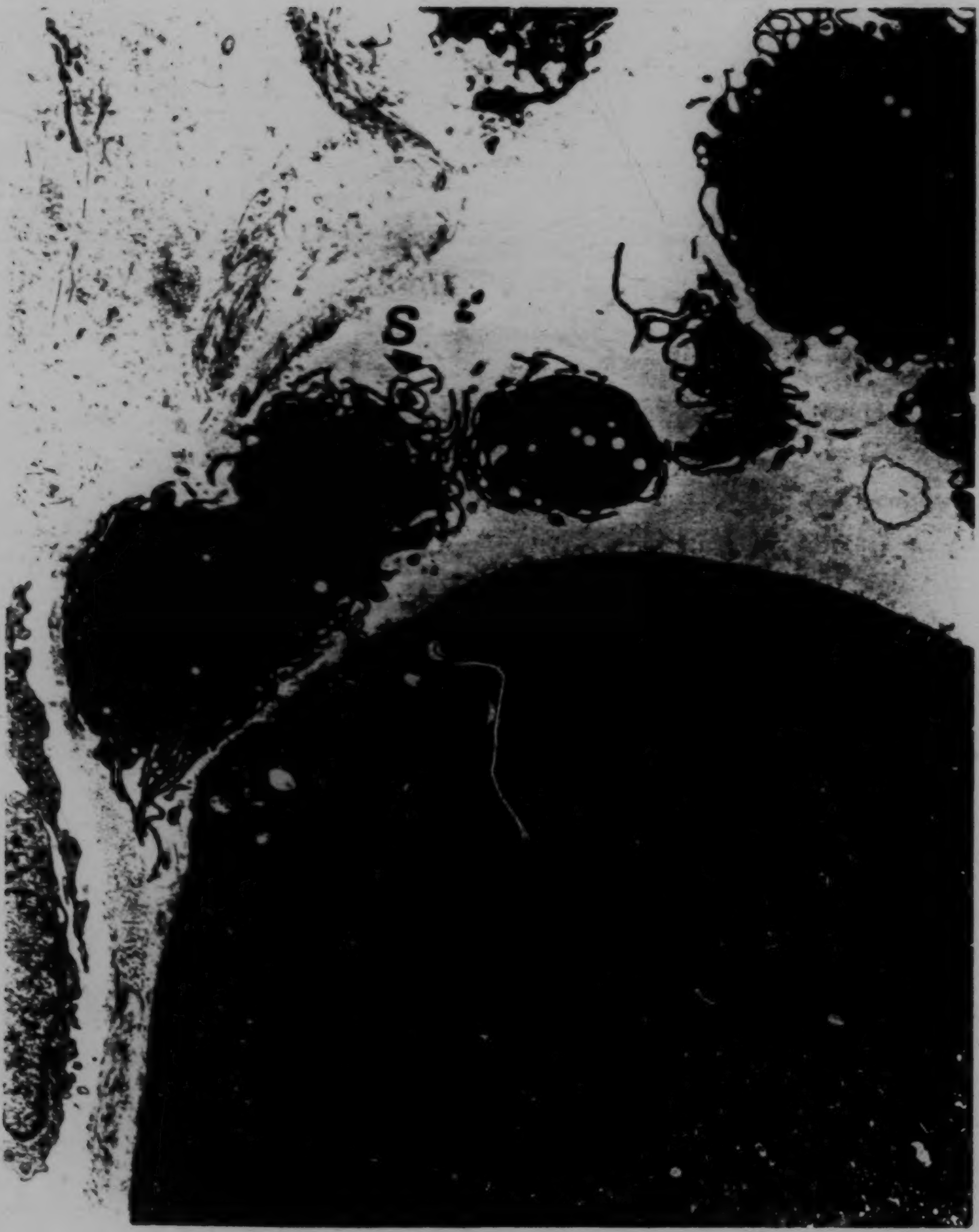

S

Figure 4. Transmission electron micrograph of gastrocnemius muscle from the injured site, 2 days post-trauma.

B. Large membraneous whorls (W) lying beneath the basal lamina of a muscle cell (Bm). Myonucleus (N) (X2950).

76A

Figure 4. Transmission electron micrograph of gastrocnemius muscle from the injured site, 2 days post-trauma.

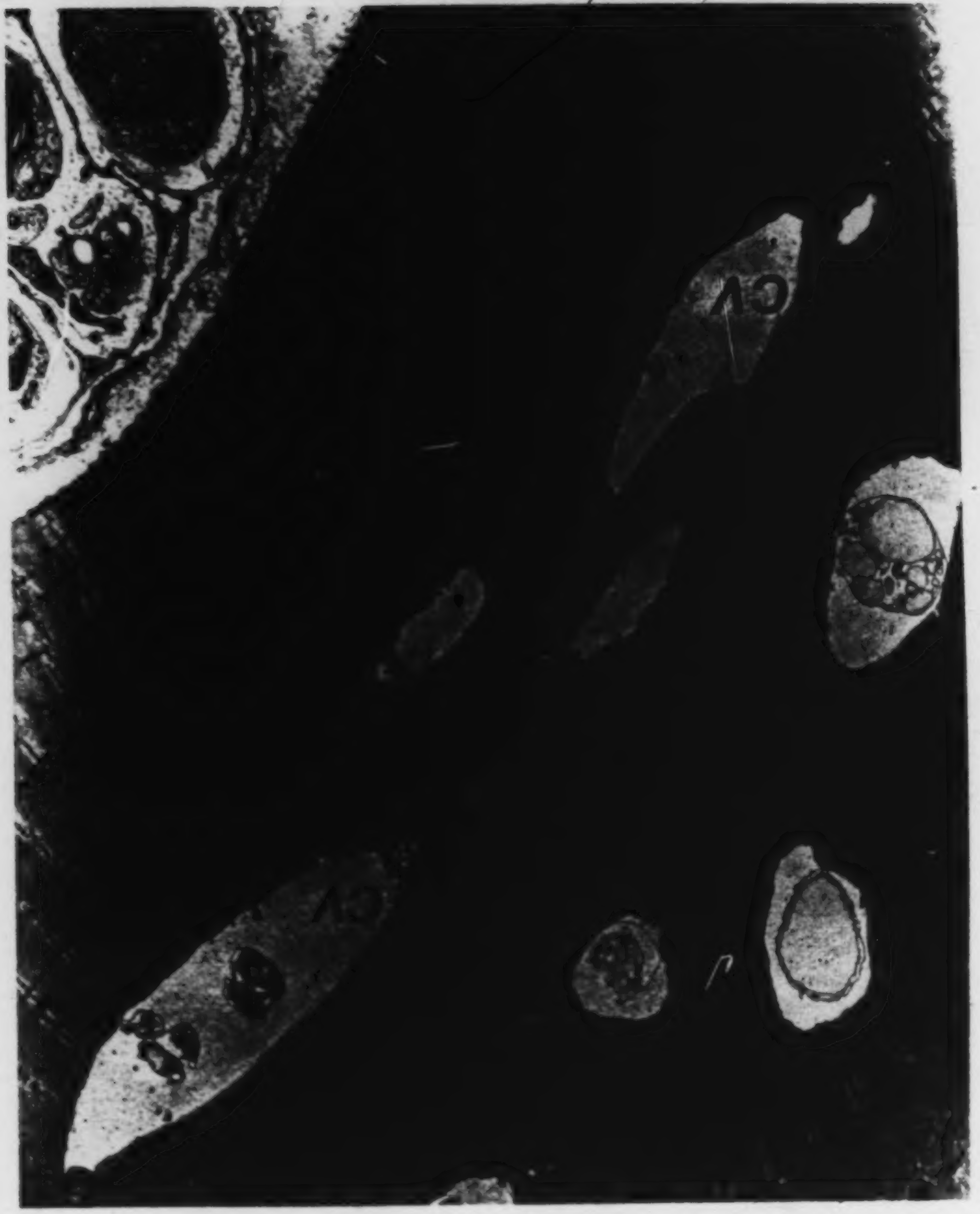
C. Vacuoles (V) and membraneous whorls (W) within a muscle cell: Myonucleus (N). (X5940).

V

Figure 4. Transmission electron micrograph of gastrocnemius muscle from the injured site, 2 days post-trauma.

D. Large clear vacuoles (CV) some of which contained amorphous material throughout the muscle fibre. A partial cross section of an intramuscular nerve (N) is shown (X 2145).

78A



2

Figure 5. Transmission electron micrograph of gastrocnemius muscle from the site of injury, 3 days post-trauma.

A. A fibroblast (Fb) with extensive endoplasmic reticulum (Er) surrounded by deposits of collagen fibrils (C) (X3135).

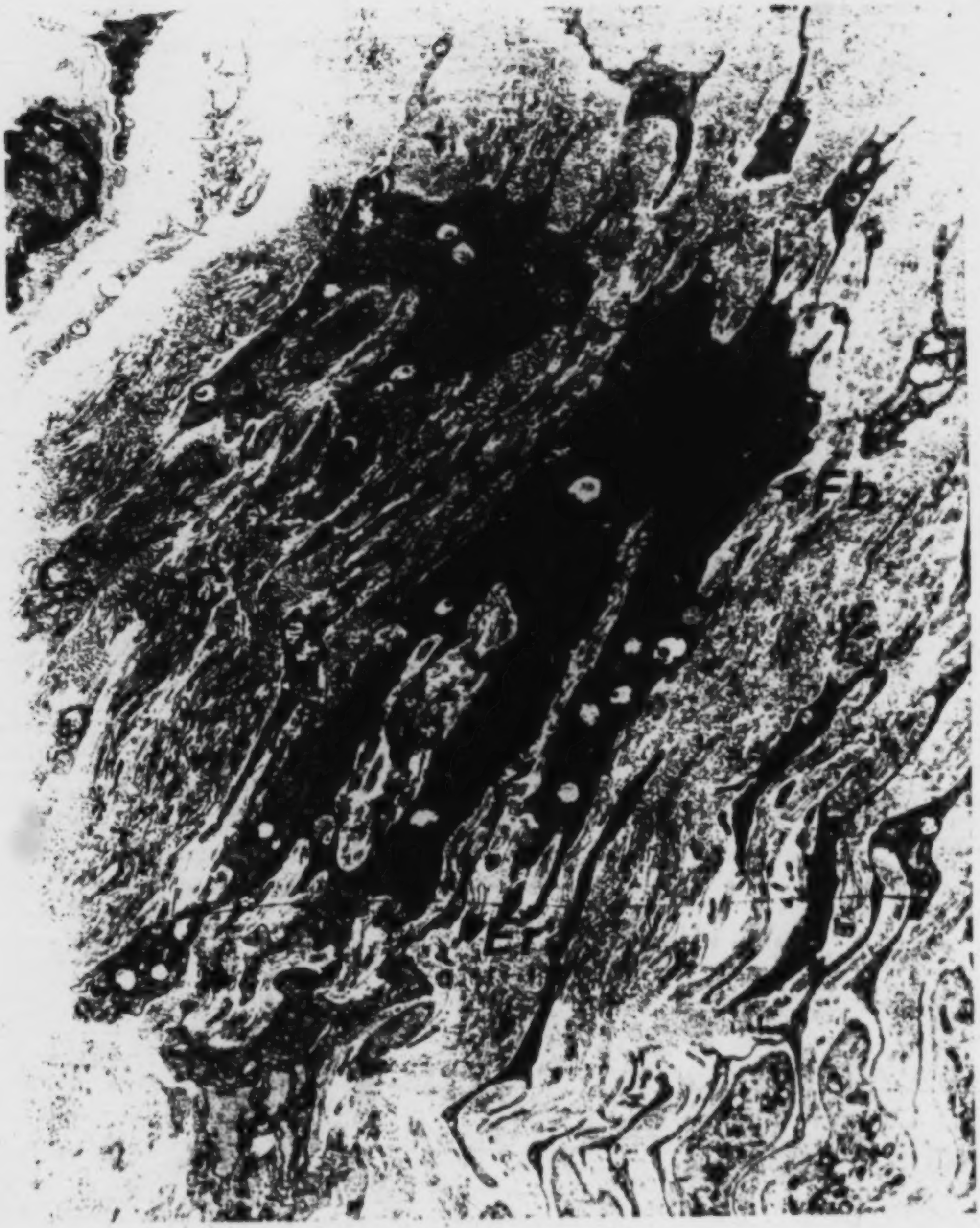

Fb
Er

Figure 5. Transmission electron micrograph of gastrocnemius muscle from the site of injury, 3 days post-trauma.

B. Two adjacent muscle cells. One muscle cell shows two myonuclei (N). A satellite cell (SA), within the basal lamina, but delimited by its plasma membrane (PM) is seen. The intermuscular space contains collagen (C) fibrils. An adjacent muscle cell (M2) shows Z-line streaming (ZLS) (X 1650).

80A

Figure 6. Transmission electron micrograph of gastrocnemius muscle from the injured site 6 days, post-trauma.

A: Fibroblasts (Fb) with extensive endoplasmic reticulum (Er) surrounded by collagen fibres (C) (X 1980).

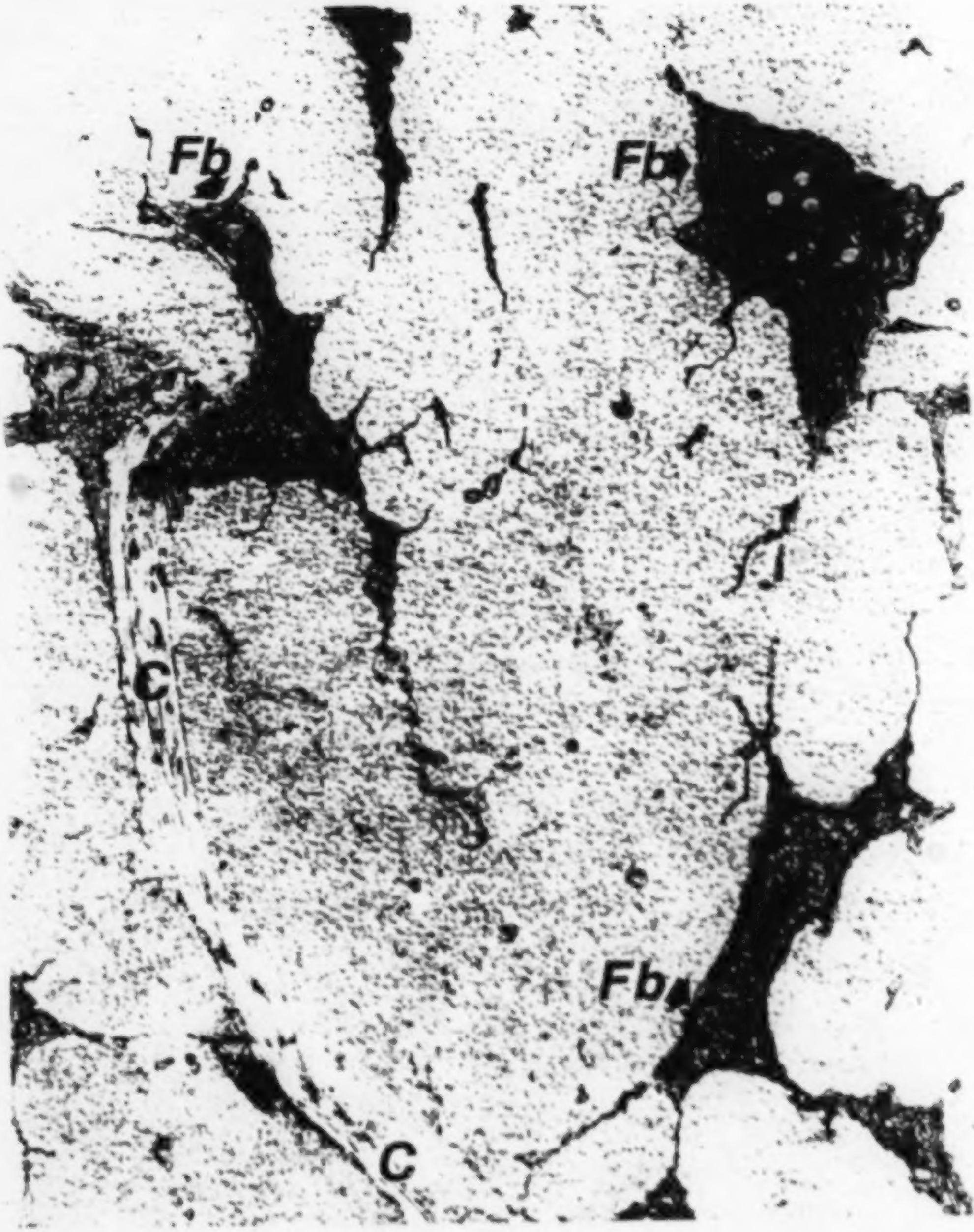
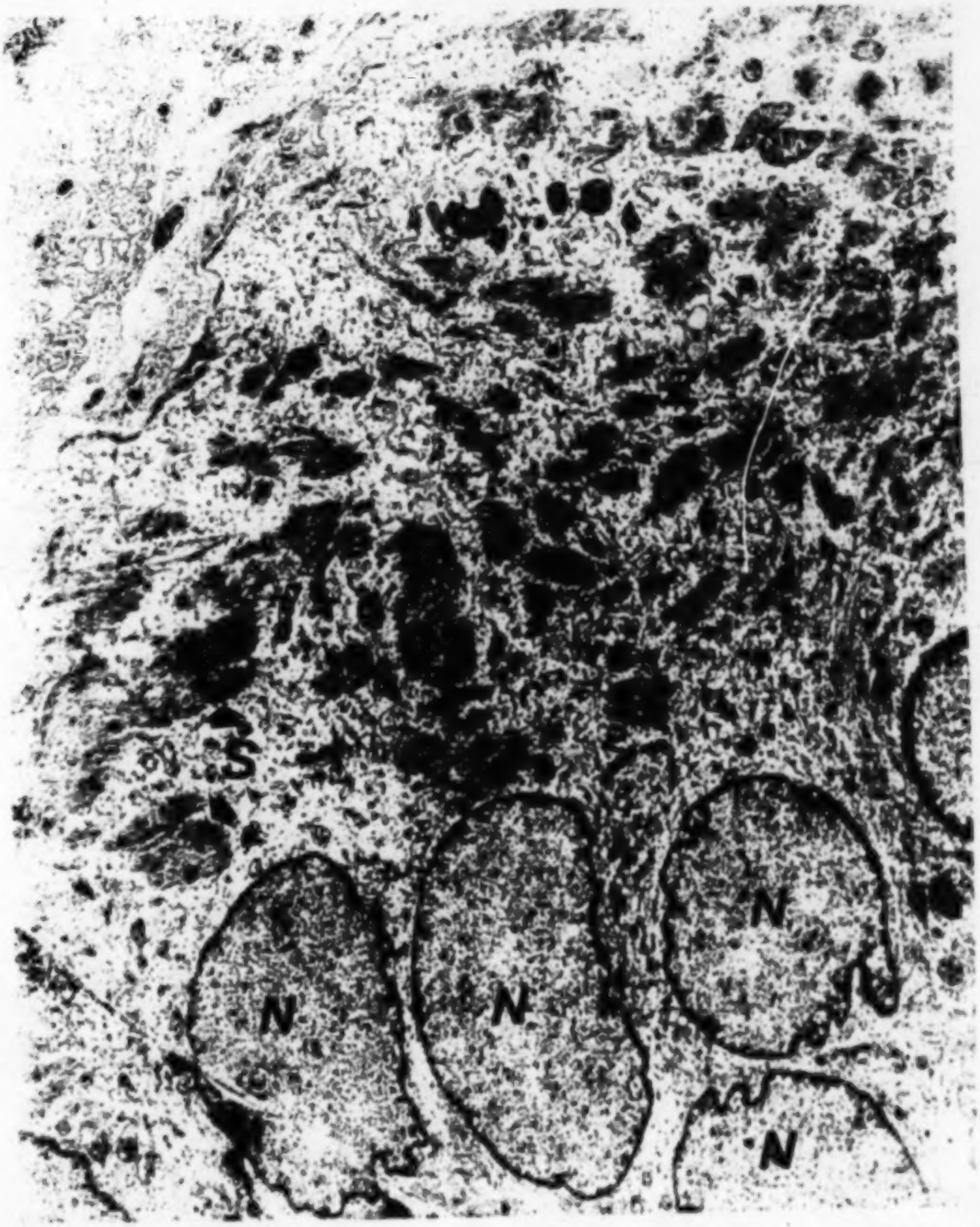

Fb
Fb
C
Fb
C

Figure 6. Transmission electron micrograph of gastrocnemius muscle from the injured site, 6 days, post-trauma.

B. Regenerating muscle cell showing multiple large vesicular nuclei (N). Newly forming sarcomeres (S) some of which contained Z-lines (Z) were observed. (X2970).


S
N
N
N
N

D. Discussion

The present results demonstrate a simple, reproducible, and humane experimental model of blunt trauma to skeletal muscle. The protocol used a device and procedure previously reported by Stratton and coworkers 1984) and was intended to produce an injury approximating the clinical lesion of a muscle contusion of moderate severity. The reproducibility of the injury was verified by determination of tissue protein content, which was highly consistent within groups and time points. The protein data agreed temporally very closely with the ultrastructural observations. During the first three days after injury when protein was extensively catabolized, evidence of phagocytosis and intracellular protein degradation was seen. During the period of protein repletion, evidence of muscle regeneration such as multiple nuclei and satellite cells were seen. This protocol may offer some advantages compared to other models of muscle injury (Allbrook et al. 1966, Allbrook 1962, Gutmann et.al. 1978, Jarvinen 1975, Le Gross Clark 1946, Schultz et al 1985, Stratton et al. 1984, Tischler et al. 1983). We obtained reproducible results using a relatively simple device compared to other models, and which does not require surgical techniques (Allbrook et al. 1966, Gutmann et al. 1978, Le Gross Clark 1946, Schultz et al. 1986). From the perspective of animal welfare, it is valuable to obtain a measurable response without inflicting severe damage. For example Stratton and coworkers (1984) used a similar device which delivered 5.87 newton-metres / cm^2, compared to 0.57 used here. In our study, significant damage was seen; this was suprising in view of the lack of overt signs of pain or altered mobility in the rat. Finally, unlike injuries produced by crushing or repeated pounding (Allbrook et al. 1966, Gutmann et al 1978, Le Gross Clark 1946, Schultz et al. 1985, Tischler et al. 1983), blunt trauma may be typical of certain sports injuries.

In the present study, we have attempted to calculate the force and momentum of impact of the device used; this information may be used to compare our device with those of others, and possibly to the force of projectiles which result in accidental injury in the field. It should be borne in mind that these values were parameters relating to the injury device and do not account for the properties of the tissues which receive the blow. The measurement of force dissipation within the muscle tissue is relevant but difficult to measure practically. This might also vary depending on the state of the muscle (e.g. relaxed vs. contracted) (Garrett et al. 1987).

Various cellular components of the muscle tissue were altered after injury with our device. These included resident cell types and infiltrating cells. The cellular components released from mechanically disrupted muscle cells and small blood vessels provided strong chemotactic stimuli for the influx of inflammatory cells into the zone of damage (Woolf 1986). Thus, at 1-2 days after trauma, inflammation appeared to be fully established in the injured gastrocnemius muscle. This period was characterized by large numbers of mononuclear cells both in the endomysial connective tissue and within some damaged muscle fibres. Intracellular and extracellular accumulations of mononuclear cells are typical of a variety of muscle injuries and myopathies (Carpenter et al 1984, Engel et al. 1986). These mononuclear cells may include tissue macrophages that were previously present, and monocytes which have been attracted to the site of injury and have crossed the vascular wall to become tissue macrophages (Woolf 1986). As well, the mononuclear cells include some B-lymphocytes and cytotoxic T-lymphocytes (Engle et al. 1986, Woolf 1986).

Mononuclear phagocytes, when stimulated, synthesize and release over 80 defined molecules, which act in an integrated fashion to mediate the anti-bacterial, anti-tumour and inflammatory activities of these cells (Adams 1984, Woolf 1986). These biologically active substances include hydrolytic enzymes (particularly proteases), metabolites of arachidonic acid (particularly prostaglandin (PG) E_2), reactive oxygen species, complement components, and a variety of polypeptide lymphokines and cytokines (cellular growth factors) (Adams 1984, Woolf 1986). Mononuclear phagocytes are involved in the removal of damaged tissue by several mechanisms (Adams 1984, Woolf 1986). These include phagocytosis and the release of hydrolytic enzymes such as proteases, or release of factors which influence the rate of catabolism within other cells. For example, PGE_2 is a potent catabolic factor for skeletal muscle cells (Rodemann et al. 1982). At the same time, cellular growth factors (cytokines) released by mononuclear phagocytes or other cells at the injured site may have a regulatory function connected with regeneration and healing. Jennische and coworkers (1987) recently demonstrated the presence of immunoreactive insulin - like growth factor - I (IGF-I) in regenerating skeletal muscle, although the cell type of origin was not identified. IGF-I is a potent anabolic factor for skeletal muscle cells.

The inflammatory response provides phagocytic cells for the removal of damaged muscle. In addition, muscle cells possess pathways of intracellular protein catabolism which may be cytosolic or lysosomal in nature (Fagan et al. 1987). Membrane-bound vacuoles, often containing amorphous material (lysosomes), were a prominent feature of the damaged muscle cells by 48 hours post-trauma. Membraneous whorls (myelin bodies) may be related to secondary lysosomes (Carpenter et al 1984), and were prevalent findings in damaged muscle in our study. Moreover, the foregoing corresponded temporally with a rapid catabolic loss of up to 27% of the muscle protein at this time.

A number of similarities may be noted between the data obtained in our study, and those reported for other types of muscle injury, which would appear to be fairly stereotyped (Allbrook 1966, Carlson 1983, Carlson 1983, Garrett etal 1987). We observed a sequential progression after injury: 1) myofibrillar disintegration and protein catabolism, 2) phagocytosis, followed by 3) regeneration, characterized by satellite cells, multiple sarcolemmal nuclei and protein repletion. The foregoing appear to correspond to the phases of intrinsic degeneration, cell mediated breakdown, and regeneration described by Carlson (1983 A,B). Similar changes may be elicited by ischemia Carlson (1983A), denervation Carlson (1983a), injection of local anesthetics (Foster et al. 1980), and injury caused by crushing (Allbrook), overuse (Armstrong), eccentric exercise (Armstrong 1986), or controlled muscle strain (Garrett et al. 1987). Allbrook et al. (1966) studied the ultrastructure of mouse muscle following crushing with hemostatic forceps. Although they observed changes which were qualitatively similar to those reported herein, the time-course of the events was extended, such that at 21 days after injury morphological abnormalities were still present.

The types of alterations seen in the present study have a variety of implications for the return to functional capacity of traumatized muscle. It is not clear to what extent the injury produced local ischemia, which can by itself cause muscle damage and is also known to impair healing (Carlson (1983A, Carlson 1983B, Solonen 1968). Similarly, limited focal crushing of motor nerves may result in functional denervation. Injured muscles may show a substantial loss of force-generating capacity (Gutmann 1978). Although this parameter was not measured in the present study, the transverse tearing of muscle fibres with myofibril retraction and major discontinuities in the sarcomeres would reduce the force with which the muscle could contract. Some large vacuoles also distorted the local myofibrillar arrays; these appeared to be derived from the sarcotubular reticulum, perhaps due to post-traumatic disturbances in fluxes of calcium and potassium ions and water (Carpenter et al 1978).

Focal interstitial fibrosis began on day three post-trauma and was increased by the day six time point. Quantitative determinations of connective tissue would be required to obtain an accurate estimate of the degree of scarring. Collagen deposition would have important implications for subsequent functional capacity of the muscle, since it involves replacement of contractile tissue, with non-contractile material. Factors influencing the relative degree of muscle cell regeneration compared to the amount of scar formation would appear to be critical in the replacement of functional muscle tissue at this stage. Finally, although muscles appeared histologically normal by day 14, complete repletion of protein and catchup to control rats was not achieved until day 21.

The specific nature and time course of the responses observed have some important clinical implications. Caution must be used in extrapolation to human injuries, however, it is possible to make some basic observations. An aim early after injury would be to protect the muscle from further damage and extension of the traumatic structural gap. Ice, compression and elevation would serve to minimize the accompanying hematoma and edema. An ideal program of therapy for muscle injury would limit destructive changes, while promoting regenerative effects on a rapid time scale. For example, an excessive inflammatory response inhibits healing (Woolf 1986). It is during this phase that non-steroidal anti-inflammatory agents may potentially have their greatest (Almekinders et al 1986, Salminen et al. 1968). Range of motion exercises may be important during healing to allow lengthening of the immature plastic scar and to minimize adhesion formation. Physical modalities such as ultrasound, laser, heat packs, interferential therapy and short wave diathermy may be useful during this period (Stratton et al 1984). Further studies to assess the effects of various currently used therapeutic modalities are required to determine their effectiveness at the cellular level. It would appear important to continue therapy until full functional capacity of the muscle tissue was attained.

In summary, a series of ultrastructural changes in muscle have been identified, as sequelae to a known, reproducible muscle crush injury in the rat. This study will form the basis for further studies on the effects of various drugs and physical modalities on the healing process. The establishment of an untreated, reproducible animal model of blunt trauma, as presented, is central to this undertaking.

REFERENCES

1. Adams, D. & T.A. Hamilton, The cell biology of macrophage activation. Ann. Rev. Immunol. 2:238-318, 1984.

2. Allbrook, D., W. de C. Baker, W.H. Kirkaldy-Willis, Muscle regeneration in experimental animals and man, J. Bone & Joint Surg. 48B:153-169, 1966.

3. Allbrook, D., An electron microscopic study of regenerating skeletal muscle, J. Anat. Lond. 96:137-152, 1962.

4. Almekinders, L.C. & J.A. Gilbert, Healing of experimental muscle strains and the effect of nonsteroidal anti-inflammatory medication, Am. J. Sports Med. 14:303-308, 1986.

5. Armstrong, R.B., Muscle damage and endurance events, Sports Med. 3:370-381, 1986.

6. Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein - dye binding, Anal. Biochem. 72:248-254, 1976.

7. Carlson, B.M. The regeneration of skeletal muscle: A review, Am. J. Anat. 137:119-150, 1983.

8. Carlson, B.M. & J.A. Faulkner, The regeneration of skeletal muscle fibres following injury: A review, Med. Sci. Sports Exerc. 115:187-198, 1983.

9. Carpenter, S., & G. Karpati, Pathology of Skeletal Muscle, Churchill Livingston, New York, 389-395, 645-652, 170-176, 1984.

10. Engel, A. & B. Banker, Myology, Basic and Clinical, McGraw-Hill, New York, 1004-1018, 1986.

11. Fagan, J.M., V.E. Baracos, A.L. Goldberg, Mechanisms of increased muscle protein breakdown during infection, in Lysosomes: Their Role in Protein Breakdown, H. Glaumann & F.J. Ballard, Eds.,Academic Press, London, 659-678, 1987.

12. Foster, A.H. & B.M. Carlson, Myotoxicity of local anaesthetics and regeneration of the damaged muscle fibres, Anaes. Analges. 58:727-736, 1980.

13. Garrett, W.E., M.R. Safran, A.V. Seaber, R.R. Glisson, B.M. Ribbeck, Biomechanical comparison of stimulated and nonstimulated skeletal muscle pulled to failure, Am. J. Sports. Med. 15:448-454, 1987.

14. Gutmann, E., B.M. Carlson, The regeneration of a hormone - sensitive muscle (levator ani) in the rat, Exp. Neurol. 58:535-548, 1978.

15. Jarvinen, M., Healing of a crush injury in rat striated muscle: a histologic study of the effect of early mobilization and immobilization on the repair process, Acta. Pathol. Microbiol. Scand. [A] 83:269-282, 1975.

16. Jarvinen, M., T. Sorvati, Healing of a crush injury in rat striated muscle: I. Description of testing a new method of inducing a standard injury in the calf muscle, Acta Pathol. Microbiol. Scand. [A] 83:259-265, 1975.

17. Jennische, E., A. Skottner, H.A. Hansson, Satellite cells express the trophic factor IGF-I in regenerating skeletal muscle, Acta Physiol. Scand., 129:9-15, 1987.

18. Keirnam, J.A., Histological and Histochemicals Methods. Theory and Practice. Pergammon Press, Oxford, 12-24, 1981.

19. Le Gross Clark, W.E. An experimental study of the regeneration of mammalian striped muscle, J. Anat. 80:24-36, 1946.

20. Mair, W., & F. Tome, Atlas of the Ultrastructure of Diseased Human Muscle, Churchill Livingstone, Edinburgh, 5-58, 1972.

21. Mastaglia, F.L., J.M. Papadimitriou, B.A. Kakulas, Regeneration of muscle in duchenne muscular dystrophy: an electron microscopic study, J. Neurol. Sci. 11:425-444, 1970.

22. Rodemann, H.P. & A.L. Goldberg, Arachidonic acid, prostaglandin E_2 and F_2 influences of protein turnover in skeletal and cardiac muscle, J. Biol. Chem. 257:1632-1638, 1982.

23. Salminen, A. & M. Kihlstrom, Protective effects of indomethacin against exercise - induced effects of injury in mouse skeletal muscle fibres, Int. J. Sports Med. 8:46-49, 1987.

24. Schultz, E., L. Jaryszak, M.C. Gibson, D.J. Allbright, Absence of exogenous satellite cell contribution to regeneration of frozen skeletal muscle, J. Muscle Res. & Cell Motility 7:361-367, 1986.

25. Schultz, E., D.L. Jaryszak, C.R. Valliere, Response of satellite cells to focal skeletal muscle injury, Muscle Nerve 8:217-222, 1985.

26. Solonen, K., L.Tarkkanen, S. Narvanen, Metabolic changes in the upper limb during tourniquet ischemia, Acta Orthop. Scand. 39:20-32, 1968.

CHAPTER III: LOCAL AND SYSTEMIC MUSCLE WASTING IN EXPERIMENTAL TRAUMA, EVIDENCE FOR A PROSTAGLANDIN-DEPENDANT MECHANISM

A. Introduction

Traumatic injury is followed by negative nitrogen balance increased urinary nitrogen loss, and a pronounced loss of skeletal muscle protein (Tischler & Fagan 1983, Birkhahan, et al. 1981, Gelfand et al. 1983, Kettelhut et al. 1988, Rennie 1985). The extent of body and tissue protein loss varies with the severity of the injury; prolonged or severe protein losses can result in cachexia (Tischler & Ragan 1983, Birkhahan et al. 1981, Gelfand et al. 1983, Kettelhut et al. 1988, Rennie 1985). The relative importance of changes in muscle protein synthesis and degradation in the muscle wasting which follows trauma has been addressed by several authors (Tischler & Fagan 1983, Birkhahan et al. 1981, Kettelhut et al. 1988). Although many hormones and factors may altered muscle protein metabolism has been suggested (Kettelhut et al. 1988, Rennie 1985), the regulation of muscle wasting remains to be fully clarified (Kettelhut et al. 1988, Rennie 1985).

Prostaglandins (PG) are cyclopentane derivatives formed from polyunsaturated fatty acids and these are released by most animal cells in response to a variety of physiological and pathological stimuli (Samuelsson et al. 1978). Skeletal muscles of humans and rats can produce metabolites of arachidonic acid including PGI_2, PGE_2, PGF_2, and thromboxane B_2 (Rodemann & Goldberg 1982, Smith et al. 1983, Nowak et al. 1983). In addition to this capability for PG production by muscle cells, mononuclear phagocytes which participate in the inflammatory response following tissue injury produce PG, particularly PGE_2 (Male et al. 1988). Recently, the possible importance of PG in the regulation of muscle protein turnover has been suggested (Kettelhut et al. 1988, Rodemann & Goldberg 1982, Smith et al. 1983, Tian & Baracos 1989,Strelkov et al. 1989). This was originally based on in vitro results, where PGE_2 appeared to be a catabolic factor which activated muscle protein degradation (Rodemann & Goldberg 1982). PGF_2 had an anabolic action in vitro by activating muscle protein synthesis (Rodemann & Goldberg 1982, Smith et al. 1983). Results from our laboratory would appear to implicate PGE_2 in the muscle wasting seen in vivo with experimental infection (Tian & Baracos, 1989) and with certain tumours (Strelkoov et al. 1989). It thus seems of interest to determine whether muscle wasting following traumatic injury may also be partly attributable to this factor. Furthermore, agents which inhibit PG synthesis have been reported to inhibit muscle protein repletion after disuse atrophy (Templeton et al. 1986). The authors of this study suggested that the anabolic action of $PGF_{2\alpha}$ may be involved in tissue regrowth in certain instances. It is unknown whether this might be the case for muscle regeneration following injury.

Whether or when PG might be important in protein metabolism subsequent to injury remains to be determined. At the same time, following soft tissue injury in sport or household accidents, non-steroidal anti-inflammatory drugs (NSAID) are frequently prescribed to control pain and the acute inflammatory response. The mode of action of these agents is by inhibition of the biosynthesis of PG and thromboxanes (Samuelsson et al. 1978, Brogden et al. 1979). It is also worthwhile to determine whether these agents have an action on muscle protein metabolism after injury, since the NSAID's might contribute to, or detract from the muscle's efficacy.

Experimental muscle injury provides a model of local regenerative events (Fisher et al. in press, 1989), as well as of the systemic metabolic response to injury (Tischler & Fagan 1983). We have recently reported a method for controlled muscle injury which produces a highly reproducible loss, and subsequent repletion of tissue protein in the rat hindlimb (Fisher et al. in the press 1989). We propose that this system may serve as a useful model for studies on the local and systemic effects of trauma, and thus, we have used it in the studies reported here.

B. Materials and Methods

Two major experiments were carried out. The first was designed to determine the effects of acute blunt trauma on muscle protein content a) locally, at the site of injury, and b) systemically, over a period of 15 days. Rats were assigned to two treatment groups, CONTROL and INJURED, such that mean initial body weight and SEM of each group were similar. Six rats per treatment per time point were used. At each time point studied, muscle protein content in the injured limb was compared with that of the uninjured contralateral limb, and with that of rats which were never injured. Statistical comparisons among rats were made using the unpaired Student's t-test; comparisons within rats were made using the paired t-test. Four muscles were studied, namely the medial gastrocnemius (MG) lateral gastrocnemius (LG), soleus (SOL) and extensor digitorum longus (EDL). These muscles were dissected whole with tendons intact, at the time of sacrifice.

A second study was designed to determine the effects of acute blunt trauma and an inhibitor of PG synthesis, naproxen (6-methoxy-a-methyl-2-napthalene acetic acid), on muscle PGE_2 production and protein content over a nine-day period following injury. Rats were assigned as described above to four principal treatment groups: CONTROL, INJURED, NAPROXEN, and NAPROXEN/INJURED. Naproxen was administered to healthy and injured rats either a) for the first 3 days immediately following injury or b) starting at 3 days and continuing until 9 days after injury. Seven rats per treatment per time point were used. The study described above was repeated twice.

Animals

All procedures were carried out in accordance with the guiding principles of the Canadian Council on Animal Care. Male Sprague Dawley rats weighting 200g (Experiment 1) and 250g (Experiment 2) were housed individually in wire cages and provided commercial rat chow (Wayne Lab Blox) and water ad libitum. Daily feed intake was determined continuously starting on the day prior to injury. Rats were kept on a 12 h light:12 h dark cycle. Body weight was recorded in each experiment at the time points indicated.

Rats were subjected to a single impact trauma to the medial aspect of the calf of the right leg, using our adaptation (Fisher et al. in the press, 1989) of the method reported by Stratton et al. (1985).

During the procedure, rats were briefly anaesthetized with halothane; control rats were also briefly anaesthetized. The trauma was delivered by dropping a solid aluminum cylinder, with a flat impact surface (1.38 cm diameter; 700g), down a tubular guide once only through a distance of 125 mm, onto the muscle. The force delivered by the device is equal to 0.57 newton-meters/cm^2; the moment of impact is equal to 1099 Kg meters/sec. This procedure has been described in detail elsewhere (Fisher et al, 1989). Animals were killed by decapitation at the time points indicated.

Measurement of PGE$_2$ Production and Protein Content

Observations of protein content and PGE$_2$ production were made as previously described (Tian & Baracos 1989, Strelkov et al. 1989). For determination of rates of PGE$_2$ synthesis, small thin samples of medial gastrocnemius muscle weighing approximately 25 mg were dissected and incubated in 3 ml Krebs-Ringer bicarbonate medium (119 mM NaCl; 4.8 mM KCl; 1.25 mM MgSO$_4$; 25 mM NaHCO$_3$; 1.24 mM NaH$_2$PO$_4$; 1.0 mM CaCl$_2$) containing 2 mM HEPES/NaOH (pH 7.4) and 0.3 ug/ml chloramphenicol. The medium also contained glucose (5 mM) and insulin (0.1 U/ml). Muscles were preincubated for 30 min at 36^{o}C, then transferred to fresh media and incubated for a further 3 h. PGE$_2$ production was estimated by radioimmunoassay of the incubation medium as described previously (Tian & Baracos 1989, Strelkov et al. 1989). The cross reactivity of the anti-PGE$_2$ antibody (ICN Immunobiologicals) with PGE$_1$ is greater than 100%, but with PGF$_{2a}$ less than 2%. Since skeletal muscle does not appear to synthesize

PGE_1 (Nowak et al. 1983), the cross reactivity would not appear to interfere with the determination of PGE_2.

To determine tissue protein content, MG, LG, SOL, and EDL muscles were dissected intact. Tissues were solublized in 1.0 N NaOH at room temperature and protein determined according to the Bradford method (Bradford, 1976). Results are expressed as total protein per muscle, or as a percent of tissue wet weight.

C. Results

Young rats, subjected to a single impact trauma to one hind limb, continued to consume feed at rates not statistically distinguishable from those of noninjured controls (Table 1). As previously described (Fisher et al 1989), injured rats did not show gross modifications of mobility such as guarding or limping. The growth rate of injured rats was not different than that of controls at any time point.

In order to assess the local and systemic effects of injury on muscle protein content, MG, LG, SOL and EDL muscles were dissected from healthy controls, as well as from both legs of the injured rats. These observations were carried out at 1, 3, 9, and 15 days after injury (Table 2A-2D). Muscles of young rats accumulated protein over a 15-day period. The extent of this net protein deposition varied with the muscle studied, and represented an increase of 49% for MG and LG, 35% for SOL, and 43% for EDL. Muscles from the injured site (e.g. the injured limb of the injured rat) showed a marked decrease in total protein within 24 h of trauma (P 0.05); this response also varied with muscle type and represented the loss of 16% of pre-existing protein in

MG, 11% in LG, 29% in SOL, and 8% in EDL. A further decrease in muscle protein content was seen at day three after trauma, such that at this time point MG, LG, SOL and EDL had lost 27%, 19%, 31% and 26%, respectively, of the protein present on day 0. After day three,

Table 1. EFFECT OF TRAUMA AND NAPROXEN ON FEED INTAKE

Cumulative Feed Intake (g rat)

Time after Trauma (d)	-1	1	2	3
Control	27.4 ± 0.9	52.4 ± 1.4	79.0 ± 2.2	104 ± 1.9
Injured	27.3 ± 0.5	52.4 ± 1.0	80.3 ± 1.4	106 ± 1.6
Naproxen	26.1 ± 0.7	52.0 ± 1.0	78.6 ± 1.2	104 ± 1.7
Injured Naproxen	27.0 ± 1.3	53.3 ± 1.2	79.9 ± 1.3	106 ± 1.5

No significant differences were observed between treatments (n=7). Rats were briefly anaesthetized with halothane. Injured rats were given a single blow to the medial aspect of the right leg. Feed intake was measured on an individual basis, starting one day prior to trauma. Naproxen was administered in the drinking water (1.6 mg / Kg body weight / day).

Table 2A. EFFECTS OF TRAUMA ON MUSCLE PROTEIN

MEDIAL GASTROCNEMIUS

Total Protein / Muscle (mg)

Time after Trauma (d)	0	1	3
Healthy Control	35.7 ± 0.9^{a}	34.5 ± 1.3^{a1}	43.1 ± 2.5^{b1}
Injured Rat			
Injured limb		30.0 ± 1.5^{b2}	26.2 ± 0.5^{c2}
Uninjured limb		36.2 ± 0.46^{a1}	33.8 ± 1.9^{a3}

Time after Trauma (d)	6	9	15
Healthy Control	48.9 ± 1.6^{c1}	51.4 ± 1.1^{c1}	52.1 ± 1.0^{c1}
Injured Rat			
Injured limb	29.2 ± 1.3^{b2}	36.1 ± 1.3^{a2}	43.1 ± 1.0^{d2}
Uninjured limb	35.8 ± 0.68^{a3}	40.2 ± 0.95^{b3}	48.2 ± 1.0^{c3}

Values with different alphabetical superscripts within the same treatment group are significantly different (P 0.05). Values with different numerical superscripts within the same time point are significantly different (P 0.05). (N-3, healthy controls; n-6, injured). Rats were briefly anaesthetized with halothane. Injured rats were given a single blow to the medial aspect of the right leg. At the time points indicated, muscles were dissected intact and solublized in 1.0 N NaOH for protein determination.

Table 2B. EFFECTS OF TRAUMA ON MUSCLE PROTEIN

LATERAL GASTROCNEMIUS

Total Protein / Muscle (mg)

Time after Trauma (d)	0	1	3	6	9	15
Healthy Control	54.3 ± 1.1^{a}	62.0 ± 2.5^{b1}	69.0 ± 1.3^{c1}	69.5 ± 0.8^{c1}	72.3 ± 0.8^{c1}	80.7 ± 1.6^{d1}
Injured Rat						
Injured limb		48.1 ± 0.9^{ab2}	44.2 ± 1.0^{c2}	49.0 ± 1.0^{db2}	62.2 ± 0.7^{e2}	66.5 ± 1.1^{f2}
Uninjured limb		49.3 ± 0.5^{b2}	46.3 ± 0.6^{c2}	49.4 ± 1.0^{b2}	63.7 ± 0.8^{d2}	68.6 ± 0.8^{c2}

Values with different alphabetical superscripts within the same treatment group are significantly different (P 0.05). Values with different numerical superscripts within the same time point are significantly different (P 0.05). (N=3, healthy controls; n=6, injured). Rats were briefly anaesthetized with halothane. Injured rats were given a single blow to the medial aspect of the right leg. At the time points indicated, muscles were dissected intact and solublized in 1.0 N NaOH for protein determination.

Table 2C. EFFECTS OF TRAUMA ON MUSCLE PROTEIN

SOLEUS

Time after Trauma (d)	0	1	3
	Total Protein / Muscle (mg)		
Healthy Control	8.20 ± 0.2^{a}	9.04 ± 0.2^{b1}	9.05 ± 0.1^{b1}
Injured Rat			
Injured limb		5.83 ± 0.2^{bd2}	5.62 ± 0.2^{b2}
Uninjured limb		6.13 ± 0.2^{b2}	6.26 ± 0.1^{b3}

Time after Trauma (d)	6	9	15
Healthy Control	9.70 ± 0.2^{b1}	9.95 ± 0.3^{b1}	11.1 ± 0.1^{c1}
Injured Rat			
Injured limb	6.71 ± 0.3^{cd2}	7.69 ± 0.2^{ae2}	8.66 ± 0.3^{af2}
Uninjured limb	6.78 ± 0.2^{b2}	7.88 ± 0.2^{ae2}	8.59 ± 0.2^{de2}

Values with different alphabetical superscripts within the same treatment group are significantly different (P 0.05). Values with different numerical superscripts within the same time point are significantly different (P 0.05). (N=3, healthy controls; n=6, injured). Rats were briefly anaesthetized with halothane. Injured rats were given a single blow to the medial aspect of the right leg. At the time points indicated, muscles were dissected intact and solublized in 1.0 N NaOH for protein determination.

Table 2D. EFFECTS OF TRAUMA ON MUSCLE PROTEIN

EXTENSOR DIGITORUM LONGUS

Total Protein / Muscle (mg)

Time after Trauma (d)	0	1	3
Healthy Control	16.0 ± 0.2^{a}	17.3 ± 0.1^{b1}	18.9 ± 0.1^{c1}
Injured Rat			
Injured limb		14.7 ± 0.3^{b2}	11.9 ± 0.2^{c2}
Uninjured limb		16.0 ± 0.1^{a3}	12.9 ± 0.3^{c2}

Time after Trauma (d)	6	9	15
Healthy Control	19.7 ± 0.1^{d1}	21.1 ± 0.1^{e1}	22.8 ± 0.3^{f1}
Injured Rat			
Injured limb	14.8 ± 0.2^{b2}	16.9 ± 0.2^{ac2}	17.9 ± 0.2^{d2}
Uninjured limb	14.6 ± 0.113	17.2 ± 0.3^{d2}	18.9 ± 0.3^{e3}

Values with different alphabetical superscripts within the same treatment group are significantly different (P 0.05). Values with different numerical superscripts within the same time point are significantly different (P 0.05). (N-3, healthy controls; n-6, injured). Rats were briefly anaesthetized with halothane. Injured rats were given a single blow to the medial aspect of the right leg. At the time points indicated, muscles were dissected intact and solublized in 1.0 N NaOH for protein determination.

Table 2E. EFFECTS OF TRAUMA ON MUSCLE PROTEIN CONTENT

21 DAYS AFTER INJURY

Total Protein / Muscle (mg)

Treatment	CONTROL	INJURED RAT INJURED LIMB	INJURED RAT UNINJURED LIMB
Medial Gastrocnemius	116 ± 0.9	113 ± 1.7	113 ± 1.4
Lateral Gastrocnemius	126 ± 4.5	124 ± 3.7	121 ± 4.6
Soleus	14.4 ± 0.5	14.4 ± 0.5	14.2 ± 0.5
Extensor digitorum longus	17.4 ± 0.4	16.4 ± 0.6	16.1 ± 0.5

No significant differences were observed between treatments for any of the muscle studied (n=5). Rats were briefly anaesthetized with halothane. Injured rats were given a single blow to the medial aspect of the right leg. At 21 days after injury, muscles were dissected intact and solublized in 1.0 N NaOH for protein determination.

muscles accumulated protein and showed a steady rate of gain. However, the total protein present in muscles of the injured limb was still significantly less than that of healthy controls at 15 days after injury. In a separate experiment, it was verified that by 21 days after injury, the protein content of injured muscles was not different from that of muscles that had never been injured (Table 2E). Thus, the muscles of the injured limb responded with a distinct catabolic phase of three days duration, followed by a prolonged period of anabolism.

Muscles in the uninjured limb of the injured rats also showed a distinct catabolic response to trauma compared to rats which were never injured (Table 2A-2D). Although highly significant, this response was always less in magnitude than that seen in the injured limb. For example, at 3 days after trauma the total protein present in MG, LG, SOL and EDL were decreased by 5%, 15%, 24% and 19% respectively, compared to the amount of protein present on day 0. This protein was also gradually replaced and at 21 days after trauma, the protein levels in these muscles was not significantly different from those of uninjured rats (Table 2E). Thus the musculature at remote sites from the injury underwent a catabolic response which varied with muscle type.

The changes seen in total protein above were seen to result from alterations in the % protein seen in the muscle tissue rather than in the absolute muscle weight. For example, there were no significant differences between the wet weights of LG muscles at any time point tested (Table 3); similar data were obtained for MG, SOL, and EDL.

Table 3. EFFECT OF TRAUMA AND NAPROXEN ON MUSCLE WEIGHT

LATERAL GASTROCNEMIUS

Time after		Muscle Weight (mg)	
Trauma (d)	0	3	9
A. Control	736 ± 8	1005 ± 11	1298 ± 24
B. Injured			
Injured Limb		1019 ± 24	1278 ± 23
Uninjured Limb			1286 ± 18
C. Naproxen			
Day 0-3		1047± 16	1274 ± 15
Day 3-9			1260 ± 8
D. Injured/Naproxen			
Day 0-3			
Injured Limb		1038 ± 16	1262 ± 15
Uninjured Limb		1059 ± 27	1262 ± 10
Day 3-9			
Injured Limb			1265 ± 18
Uninjured Limb			1263 ± 28

No significant differences were observed between treatments within time points (n=7). Rats were briefly anaesthetized with halothane. Injured rats were given a single blow to the medial aspect of the right leg. Rats were killed at the time points indicated and the medial gastrocnemius dissected intact and weighed. Naproxen was administered in the drinking water (1.6 mg / Kg body weight / day). No significant difference were observed amongst treatments within time points.

Table 4. EFFECTS OF TRAUMA ON MUSCLE PROTEIN PERCENT PROTEIN

Treatment	Muscle Protein Content (%) CONTROL RAT	INJURED RAT INJURED LIMB	INJURED RAT UNINJURED LIMB
Medial Gastrocnemius	12.7 ± 0.7^{a}	7.7 ± 0.4^{b}	9.1 ± 0.3^{c}
Lateral Gastrocnemius	14.7 ± 0.4^{a}	9.9 ± 0.2^{b}	10.3 ± 0.2^{b}
Soleus	17.5 ± 0.2^{a}	10.0 ± 0.4^{b}	11.1 ± 0.2^{c}
EDL	14.6 ± 0.3^{a}	9.5 ± 0.2^{b}	10.4 ± 0.2^{c}

Values with different alphabetical superscripts within the same muscle are significantly different ($P<0.05$). (n=3 controls; n=6 injured). Rats were briefly anaesthetized with halothane. Injured rats were given a single blow to the medial aspect of the right leg. At 3 days after trauma, muscles were dissected intact and solublized in 1.0 N NaOH for protein determination.

However, % protein was significantly reduced, compared to muscles of rats which had not been injured (Table 4). The % protein of muscles in the injured limb was reduced at day 3 by 25%, 35%, 41% and 39% in the MG, LG, SOL, and EDL, respectively. The percent protein of muscles in the uninjured limb was also reduced by 25%, 32%, 34%, and 31% in the MG, LG, SOL, and EDL, respectively, at day three after injury.

Since the time course of the catabolic and subsequent anabolic phases following trauma had been characterized in the first study only selected time points were used in the second study. The interval from the time of trauma to three days was used to characterize the catabolic phase and the interval from day 3 to day 9 used to characterize the anabolic phase. Trauma procedure had no significant effect on the growth rate of the rats or muscle weights (data not shown). The administration of naproxen in the drinking water did not influence whole body (data not shown) or muscle growth (Table 3), in accordance with our previous results (Tian & Baracos 1989, Strelkov et al. 1989). Finally, neither trauma nor naproxen had any demonstrable effect on feed intake over the 9-day period studied.

Muscle Protein: Effects of Injury

Total protein data for the four muscles studied are shown in Table 5A-D. At 3 days after trauma, all muscles in the injured limb showed a significant loss of pre-existing protein, this effect varied with muscle type (-40% MG: -24% LG; -48% SOL; -39% EDL). Muscles in the injured limb showed substantial regrowth by 9 days after injury, although at this time the total protein present was still less ($P<0.05$)

than in corresponding muscles that had never been injured. Unfortunately, in this experiment all of the protein data of the contralateral (uninjured) limb were accidentally lost for the 3 day time point. At 9 days after trauma, the protein present in the uninjured limb of injured rats was reduced ($P<0.05$); this was true of all four muscles studied. Thus, both local and systemic muscle protein loss and regrowth were observed in the second experiment.

Muscle Protein: Effects of Naproxen Treatment

When healthy control rats were treated with naproxen for either the first three days following injury or from day 3 through day 9 after injury, this agent had no significant effect on the total protein present in any of the four muscles studied (Table 5A-D). When naproxen was administered to rats which had previously been injured, a significant increase in muscle protein was observed with both protocols of administration. Naproxen suppressed the degree of muscle protein loss observed at 3 and 9 days after injury, in all four muscles of the injured limb; this suppression varied with muscle type. The greatest degree of improvement in muscle protein content was seen in EDL, followed by MG, SOL, and LG. The administration of naproxen also significantly improved the protein levels observed in muscles of the uninjured limb at 3 and 9 days after injury. This was true of all four muscles examined and also varied with muscle type in the order indicated above for muscles of the injured limb. These results demonstrate that both local muscle protein loss at the site of injury, and the systemic effect observed in the contralateral limb, appear sensitive to the administration of naproxen.

Table 5A. EFFECTS OF TRAUMA AND NAPROXEN ON MUSCLE PROTEIN

MEDIAL GASTROCNEMIUS MUSCLE

Time after Injury (d)	Total Protein / Muscle (mg)		
	0	3	9
A. Healthy control	82.7 ± 1.4	107 ± 2.4^{a}	156 ± 2.2^{a}
B. Injured Rat			
Injured limb		63.8 ± 2.5^{b}	124 ± 1.7^{b}
Uninjured limb			128 ± 0.9^{b}
C. Naproxen			
i. Day 0-3		113 ± 1.9^{a}	157 ± 2.1^{a}
ii. Day 3-9			153 ± 2.8^{a}
D. Injured/Naproxen			
i. Day 0-3			
Injured limb		75 ± 1.7^{c}	141 ± 2.0^{c}
Uninjured limb		84 ± 1.5^{d}	147 ± 2.6^{d}
ii. Day 3-9			
Injured limb			138 ± 2.3^{cd}
Uninjured limb			146 ± 2.9^{cd}

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^{a,b,c,d} values in the same column having different superscripts are different, ($p<0.05$; n=7). Rats were given a single impact trauma to the left medial aspect of the lower limb (Injured) or were not injured (Control). Naproxen was given for either days 0 through 3, or days 3 through 9 following trauma. In traumatized rats, both the injured leg and uninjured leg were studied.

Table 5B. EFFECTS OF TRAUMA AND NAPROXEN ON MUSCLE PROTEIN

LATERAL GASTROCNEMIUS MUSCLE

Time after Injury (d)	Total Protein / Muscle (mg) 0	3	9
A. Healthy control	122 ± 1.8	148 ± 3.7^{a}	228 ± 3.9^{a}
B. Injured Rat			
Injured limb		113 ± 1.2^{b}	174 ± 2.4^{b}
Uninjured limb			189 ± 1.6^{c}
C. Naproxen			
i. Day 0-3		153 ± 1.8^{a}	228 ± 2.2^{a}
ii. Day 3-9			227 ± 1.7^{a}
D. Injured/Naproxen			
i. Day 0-3			
Injured limb		124 ± 1.6^{c}	182 ± 1.4^{d}
Uninjured limb		132 ± 1.7^{d}	187 ± 1.7^{c}
ii. Day 3-9			
Injured limb			193 ± 1.2^{e}
Uninjured limb			194 ± 1.2^{e}

a,b,c,d values in the same column having different superscripts are different, ($p<0.05$; $n=7$). Rats were given a single impact trauma to the left medial aspect of the lower limb (Injured) or were not injured (Control). Naproxen was given for either days 0 through 3, or days 3 through 9 following trauma. In traumatized rats, both the injured leg and uninjured leg were studied.

Table 5C. EFFECTS OF TRAUMA AND NAPROXEN ON MUSCLE PROTEIN

SOLEUS MUSCLE

Time after Injury (d)	Total Protein / Muscle (mg) 0	3	9
A. Healthy control	15.2 ± 0.5	19.3 ± 0.6[a]	29.0 ± 0.9[a]
B. Injured Rat			
Injured limb		10.0 ± 0.7[b]	15.6 ± 0.5[b]
Uninjured limb			17.4 ± 0.3[b]
C. Naproxen			
i. Day 0-3		21.0 ± 0.2[a]	29.1 ± 0.7[a]
ii. Day 3-9			31.0 ± 0.5[a]
D. Injured/Naproxen			
i. Day 0-3			
Injured limb		14.3 ± 0.4[d]	19.9 ± 0.1[c]
Uninjured limb		15.0 ± 0.4[d]	22.4 ± 0.7[d]
ii. Day 3-9			
Injured limb			18.2 ± 0.3[c]
Uninjured limb			21.0 ± 0.6[d]

[a,b,c,d] values in the same column having different superscripts are different, ($p<0.05$; n=7). Rats were given a single impact trauma to the left medial aspect of the lower limb (Injured) or were not injured (Control). Naproxen was given for either days 0 through 3, or days 3 through 9 following trauma. In traumatized rats, both the injured leg and uninjured leg were studied.

Table 5D. EFFECTS OF TRAUMA AND NAPROXEN ON MUSCLE PROTEIN

EXTENSOR DIGITORUM LONGUS

Time after Injury (d)	Total Protein / Muscle (mg) 0	3	9
A. Healthy control	13.2 ± 0.3	23.0 ± 0.3^a	30.4 ± 0.7^a
B. Injured Rat			
Injured limb		14.0 ± 0.3^b	24.5 ± 0.3^b
Uninjured limb			25.4 ± 0.4^b
C. Naproxen			
i. Day 0-3		23.0 ± 0.3^a	31.9 ± 0.9^a
ii. Day 3-9			33.0 ± 0.5^a
D. Injured/Naproxen			
i. Day 0-3			
Injured limb		18.2 ± 0.3^c	29.5 ± 0.3^a
Uninjured limb		19.2 ± 0.5^d	31.5 ± 0.1^a
ii. Day 3-9			
Injured limb			30.1 ± 0.6^a
Uninjured limb			30.0 ± 0.6^a

a,b,c,d values in the same column having different superscripts are different, ($p<0.05$; $n=7$). Rats were given a single impact trauma to the left medial aspect of the lower limb (Injured) or were not injured (Control). Naproxen was given for either days 0 through 3, or days 3 through 9 following trauma. In traumatized rats, both the injured leg and uninjured leg were studied.

Table 6. EFFECTS OF TRAUMA ON PGE_2 PRODUCTION BY MEDIAL GASTROCNEMIUS MUSCLE

	PGE_2 Synthesis (pg PGE_2 / mg muscle / 3 h)		
Time after Trauma (d)	0	3	9
A. Healthy control	10.5 ± 3.4^{a}	10.3 ± 1.4^{a}	11.4 ± 1.6^{a}
B. Injured Rat			
Injured limb		18.9 ± 2.8^{b}	14.1 ± 1.6^{a}
Uninjured limb		17.3 ± 2.5^{b}	9.3 ± 3.6^{a}
C. Naproxen			
Day 1-9		9.9 ± 1.1^{a}	6.0 ± 1.4^{c}
Day 3-9			8.7 ± 1.7^{ac}
D. Injured/Naproxen			
Day 1-9			
Injured limb		13.2 ± 1.9^{a}	11.4 ± 1.6^{a}
Uninjured limb		10.5 ± 1.7^{a}	11.8 ± 1.0^{a}
Day 3-9			
Injured limb			13.3 ± 2.7^{a}
Uninjured limb			11.7 ± 1.8^{a}

Values with different alphabetical superscripts are significantly different (P 0.05; $n=5$). Rats were briefly anaesthetized with halothane. Injured rats were given a single blow to the medial aspect of the right leg. Over the time intervals indicated, animals were administered naproxen (1.6 mg / Kg body weight /day).

Muscle PGE_2 Production: Effects of Trauma and Naproxen

Biopsies of medial gastrocnemius muscles from healthy control rats produced 10 to 11 pg of PGE_2 per mg wet weight during 3 h of incubation (Table 6). These results are in accordance with those obtained previously in intact rat epitrochlearis muscles (Tian & Baracos 1989, Strelkov et al. 1989). Despite the limitations of using muscle pieces in vitro, the samples appear to give representative results for PGE_2 synthesis. Significantly, increased PGE_2 production was observed at day 3 in MG from both the injured and uninjured limbs; this effect was not evident at 9 days. Although the oral administration of naproxen did not affect basal levels of PGE_2 synthesis, the augmented PGE_2 production associated with the trauma was inhibited by this agent.

D. Discussion

The present results demonstrate local and systemic muscle protein catabolism following trauma in a simple experimental model. The procedure is reproducible, and does not appear to be complicated by gross modifications of feed intake or mobility, two important factors which by themselves affect muscle protein metabolism (Kettelhut et al. 1988). Although the injury appeared minimal as judged by feed intake, growth, and behavior, it was sufficient to give a clear catabolic response of the musculature both at the site of injury and at remote sites. The establishment of this model in the present and previous studies (Fisher et al. 1989) will serve as a basis for studies of protein metabolism in trauma and of the effects of various modalities on the healing process. Tischler and Fagan (1983) demonstrated alterations in muscle protein, amino acid and carbohydrate metabolism both locally and systemically using a similar model employing a multiple-blow trauma device.

Over a 3-day period after injury, the net loss of pre-existing protein was observed in four muscles in the injured limb as well as the same muscles in the uninjured limb. The large number of muscles studied near to and distant from the site of injury demonstrates the variable nature of the response. Some of the muscles of the injured limb are within the zone of impact, including the MG, LG, and SOL. The EDL of the injured limb was never directly injured because of its position on the opposite side of the tibia, but also showed a marked catabolic response. Some of the differences between muscles of the uninjured limb may be attributable to their fibre type, or alternatively, to their relative growth rates and synthetic capacity at the time of trauma. The catabolic response was always greatest in the soleus, a largely slow twitch muscle in the rat; the muscle was also the slowest growing at the time of injury.

Biochemical changes following muscle injury are not well-characterized, but like many other instances of inflammation, muscle injury is likely to involve the systemic and local production of prostaglandins (Male et al. 1988). The procedure used in the present study would appear to have provoked an acute inflammatory response, as judged by the production of PGE_2 by samples of muscle isolated at 3 days after injury. Increased PGE_2 production was evident both in the MG which was directly traumatized, and in the same muscle in the contralateral limb. The enhanced PGE_2 in the injured limb may have come from activated tissue macrophages in the course of the acute inflammatory response (Male et al. 1988). It remains unclear as to which factor(s) might provoke PGE_2 synthesis in muscles remote from the site of injury.

Naproxen is known to inhibit the production of prostaglandins by the inhibition of cyclo-oxygenase activity (Brodgen et al. 1979). In the present study, this agent inhibited muscle PGE_2 synthesis when administered orally, as shown previously (Tian & Baracos 1989, Streldov et al. 1989). The ability of this agent to partially inhibit muscle protein loss following injury suggests that metabolites of the cyclo-oxygenase reaction may directly or indirectly be responsible for alterations in muscle protein metabolism following injury. This result agrees with our observation that muscle protein loss induced by infection and certain tumours (Tian & Baracos 1989, Strelkov et al. 1989) is sensitive to inhibition by the same agent. In the case of infection and trauma, this result is consistent with the presence of an inflammatory response.

NSAID are widely used after minor soft tissue injury to control acute inflammation and pain (e.g. Salminen & Kihlstrom 1987, Almekinders & Gilbert 1986, Dahners et al. 1988), although the influence of these agents on subsequent healing and function have not been extensively studied. In our study, naproxen appeared to inhibit the acute catabolic loss of muscle protein and to enhance the rate of tissue protein replacement. Further studies are required to determine whether the protein composition of the healed limb is identical to that of muscles which were never injured, and whether the functional capacity (e.g. strength) is eventually recovered. Since our prior ultrastructural study showed some qualitative evidence for scar formation, it would be particularly interesting to determine the ratio of collagen:myofibrillar protein in the injured muscles after complete protein repletion.

Treatment of muscle injuries is often based in empirical findings. Amongst the common treatments are heat, ultrasound, immobilization, mobilization, steroids and non-steroidal anti-inflammatory medication. The NSAIDS are used particularly because of their analgesic properties. Most recently, some efforts have been made to assess the effects of NSAID treatment on tissue functional characteristics after injury, such as the mechanical strength of injured muscle (Almekinders & Gilbert 1986) and tendon (Dahners et al. 1988), or on such parameters as histological appearance (Salminen & Kihlstrom 1987) or rates of muscle protein repletion (Templeton et al. 1986). In different experiments NSAID have been shown to adversely affect (Templeton et al. 1986, Almekinders & Gilbert 1986), or improve (Salminen & Kihlstrom 1987, Dahners et al. 1988) different parameters. The effects may depend upon the injury model used, the timing of NSAID application relative to injury, and the tissue examined. Without a combined analysis of the effects of NSAIDs on the healing and subsequent function of muscle and supporting tissues, it is as yet difficult to make specific recommendations for their use.

References

1. Almekinders, L.C., Gilbert, J.A., 1986 Healing of experimental muscle strains and the effects of non steroidal anti-inflammatory medication. Am. J. Sports Med. 14:303-308.

2. Birkhahan, R.H., Long, C.L., Fitkin, D., Jeevanandam, M., Blakemore, W.S., 1981, Whole body protein metabolism due to trauma in man as estimated by L-(^{15}N) alanine, Am. J. Physiol. 241:E64.

3. Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein dye binding, Anal. Biochem. 72:248-254.

4. Brogden, R.N., Heel, R.C., Speight, T.M., Avery, G.S., 1979, Naproxen up to date: a review of its pharmacologic properties and therapeutic efficacy and use in rheumatic disease and pain states, Drugs 18: 241-277.

5. Dahners, L.E., Gilbert, J.A., Lester, G.E., Taft, T.N., Payne, L.Z., 1988 The effect of a non steroidal anti-inflammatory drug on the healing of ligaments, Am.J. Sports Med. 16:641-646.

6. Fisher, B.D., Baracos, V.E., Shnitka, T.K., Mendryk, S.W., Reid, D.C., 1989, Ultrastructural events following acute muscle trauma, Med. Sci. Sports Exer (in the press).

7. Gelfand, R.A., Defronzo, R.A., Gusberg, R., 1983, Metabolic alterations associated with major injury or infections, in New Aspects of Clinical Nutrition, G. Kleinberger, E. Deutsh eds, Karger, Basel.

8. Kettelhut, I., Wing, S., Goldberg, A.L., 1988, Endocrine regulation of protein breakdown in skeletal muscle, Diab. Metab. 4:751-772.

9. Male, D, Champion, B., Cooke, A., 1988, Immunology, MedSci/McGraw-Hill, New York.

10. Nowak J., Bohman, S.O., Alster, P., Berlin, T., Cronenstrand, R., Sonnenfeld, T., 1983, Biosynthesis of prostaglandins in microsomes of human skeletal muscle and kidney, Prost. Leuk. Med. 11:269-279.

11. Rennie, M.J., 1985, Muscle protein turnover and wasting due to injury and disease, Brit. Med. Bull. 41:257-264.

12. Rodemann, H.P., Goldberg, A.L., 1982, Arachidonic acid, prostaglandin E_2 and $F_{2\alpha}$ influence rates of protein turnover in skeletal and cardiac muscle, J. Biol. Chem. 257:1632-1638.

13. Salminen, A., Kihlstrom, M., 1987, Protective effect of indomethacin against exercise induced injuries in mouse skeletal muscle fibres, Int. J. Sports Med. 8:46-49.

14. Samuelsson, B., Granstrom, G.E., Hamberg, M., Hammarstrom, S., Malmster, C., 1978, Prostaglandins and thromboxanes. Ann. Rev. Biochem. 47:907-1029.

15. Smith, R.H., Palmer, R.M., Reeds, P.J., 1983, Protein synthesis in isolated rabbit forelimb muscle, Biochem. J. 214:153-161.

16. Stratton, S.A., Heckmann, R., Francis, R.S., 1985, Therapeutic ultrasound, its effects on the integrity of a non-penetrating wound J. Orthoped. Sports Phys. Therap. 5:278-281.

17. Strelkov, A.B.S., Fields, A.L.A., Baracos, V.E., 1989, Effects of systemic inhibition of prostaglandin synthesis on protein metabolism in tumour-bearing rats, Am. J. Physiol. 257:261-269.

18. Templeton, G.H., Padalino, M., Moss, R., 1986, Influences of inactivity and indomethocin on soleus phosphotidlethanolamine and size Prostaglandin. 31:545-550.

19. Tian, S., Baracos, V.E., 1989, Prostaglandin-dependant muscle wasting in the broiler chick (Gallus domesticus) and laboratory rat (Rattus norvegicus), Biochem. J. 263:485-490.

20. Tischler, M.E., Fagan, J.M., 1983, Response to trauma of protein, amino acid and carbohydrate metabolism in injured and uninjured rat skeletal muscle, Metabolism 32:853-868.

CHAPTER IV: EFFECTS OF THERAPEUTIC ULTRASOUND IN SKELETAL MUSCLE FOLLOWING ACUTE BLUNT TRAUMA

A. INTRODUCTION

In sport, soft tissue injury resulting from a single blow or crush is common (Arnheim 1989) and ultrasound therapy is frequently used (Reilly 1981). The availability of therapeutic ultrasound has made this one of the most commonly used physical modalities in sports medicine today , and clinical dosages often remain unmodified irrespective of the tissue composition or type of injury (Welsh & Sheppard 1986).

Much of the information directly relating to the effects of ultrasound on soft tissue lesions is based on macroscopic observations (Lehmann et al.1967, Stratton et al.1984, Griffin 1966, Dyson & Suckling 1978), and few studies focus directly on the cellular responses of injured tissue following the application of ultrasound (Stratton et al.1984). One study suggested that ultrasound enhances protein synthesis in fibroblasts (Harvey et al.1975). Others reported that ultrasound stimulated the rate of growth of replacement tissue at the site of pressure sores (Dyson & Suckling 1978). Paul et al. (1960) found that ultrasound promoted healing of pressure sores by stimulating the production of granulation tissue in trophic ulcers. Ultrasound has also been reported to reduce inflammation by directly inducing mast cells to release histamine which in turn produces vasodilation and increased vascular permeability (Fyfe & Chahl 1984). In a separate experiment, the same authors also injected silver nitrate into the rat's hind paw and examined the effects of various ultrasound frequencies on plasma extravasation. They reported that ultrasound decreased plasma extravasation (Fyfe & Chahl 1980). Cortisol, which is known for its strong anti-inflammatory effects, was also found to increase in plasma concentrations following the application of ultrasound. Griffin (1966) reported that, following sonaction over nerve plexes and peripheral nerves, the production of cortisol increased resulting in decreased pain and inflammation.

Prostaglandin synthesis is a well documented feature of inflammation. Prostaglandin E_2 is present in inflammatory exudates and is released from macrophages during the acute inflammatory response (Davis & Shires 1986). This factor appears to modify tissue protein metabolism, and data presented in Chapter III suggest that PGE_2 is an important catabolic factor in muscle injury.

The reports on the effects of ultrasound on prostaglandin production are conflicting. Reid (1981) noted that the literature on the anti-inflammatory potential of ultrasound on prostaglandin production is confusing, and the results of a number of other studies are unsatisfactory when evaluating the statistical results of clinical trials. Despite this negative assessment of the anti-inflammatory potential of ultrasound, Reid (1981) reported that when ultrasound is delivered at low dosages for certain pathological conditions, a potent anti-inflammatory effect is achieved and prostaglandin production is reduced.

The literature pertaining to the effects of ultrasound on wound healing following trauma is well established (Griffin 1966, Reilly 1981, Fyfe & Chahl 1984, Stratton et al. 1984). However, the effects of ultrasound on skeletal muscle and various inflammatory mediators following acute blunt trauma is less well understood (Goddard et al.1983). It is not presently clear how the application of ultrasound alters the production of prostaglandins during the acute inflammatory response or how ultrasound affects muscle protein content following acute blunt trauma. The objective of these studies was to clarify the effects of therapeutic ultrasound on the foregoing parameters.

B. MATERIALS AND METHODS

Animals and Diets

All the procedures described below were carried out in accordance with the guiding principles for the care and use of laboratory animals of the Canadian Council on Animal Care. Forty female Sprague-Dawley rats, weighing 180-230 g. at the start of the experiment, were housed in groups of four in wire cages and provided with commercial rat chow (Wayne Lab Blox) and water *ad libitum*. Rats were maintained on a 12 h light: 12 h dark cycle.

Experimental Trauma

Rats were subjected to a single impact trauma to the medial aspect of the gastrocnemius of the right leg, using an adaptation of a method reported by Stratton et al. (1984). During this procedure, rats were briefly anaesthetized with halothane. The medial side of the calf muscle was padded with a layer of gauze (0.5 cm thick) to prevent tearing of the skin. The trauma was carried out by dropping a solid aluminum cylinder (1.38 cm in diameter x 27 cm in length, weighing 700 g) once only through a distance of 125 mm, onto the padded muscle. The force delivered by the falling object with these characteristics was calculated to equal 0.57 newton - meters/cm^2. To ensure stabilization and accurate delivery, the cylinder was dropped down a tubular guide fixed to a ring stand. The limb was positioned manually with the foot stabilized at a 90 degree angle to the tibia. The location of the large belly of the gastrocnemius muscle was determined by palpation and the device was placed over this area. The tibia was protected from accidental fracture by carefully placing it out of the line of travel of the device. The site of skin impact was marked with indelible ink at the time of trauma to ensure subsequent localization. Healthy controls were sham-treated, including brief anaesthesia with halothane.

Ultrasound Treatment

The area to be insonnated was shaved of fur. Ultrasound treatment was given from day 3 to day 9 twice daily (1.5 watts/cm^2 x 3 min x 6 day). The treatment was applied under demineralized water using a plastic water bath to minimize scattering. The foot of the rat was maintained in extension by gentle traction on the distal phalanges, the legs were widely separated and the right leg serving as control for the treatment, was positioned high and out of the water to minimize the likelihood that it might be affected by sideways scatter of the ultrasound from the transducer (Fyfe & Chahl 1980). The ultrasound was administered with a Siemens Sonostat ultrasound unit with a frequency of 870 kHz. The ultrasound machine was calibrated at the beginning and at the midpoint of the study; the readings were found to be identical. During underwater insonnation of each rat, the transducer was parallel to the injured area and was moved completely across the left medial aspect of the lower limb at a distance of approximately 1 cm from the treatment site. All control rats were treated in the same manner asthose rats that received ultrasound except that the sound head was not activated during the sham ultrasound treatment.

Experimental Design

As seen previously (Chapter II, Chapter III), acute blunt trauma was followed by muscle protein loss for 3 days, then by a progressive muscle protein repletion. In this study we chose to observe the effects of therapeutic ultrasound applied for 6 days (day 3 - day 9 after injury) representing the period of regeneration and muscle protein repletion.

Four major treatment groups were employed: I. Healthy, uninjured controls were sacrificed on days 3 and 9. II. A second control group received ultrasound therapy as described above, but were not injured. This group was sacrificed on day 9. III. Traumatized rats received a single impact trauma to the medial aspect of the right leg. Traumatized rats were sacrificed on days 3 and 9 after injury and muscles from both the injured leg and the contralateral, uninjured leg were dissected. IV. Some traumatized rats also received ultrasound therapy as described above, and were sacrificed on day 9.

Rats were allocated to treatments by initial body weight, such that the mean and standard error of the mean for each group were similar. Six rats were allocated to each treatment and time point. Data were analyzed using the unpaired Student's T-test. Traumatized muscles and the contralateral untraumatized muscle within the same animal were compared using a paired T-test.

i. Measurement of PGE_2 Production and Protein Content

Observations of protein content and PGE_2 production were made on days 3 and 9 after trauma. In the traumatized rats, at day 3 and day 9 both the trauma and uninjured leg (trauma control) were studied. Muscle biopsies (25 mg) were incubated in 3 ml Krebs-Ringer bicarbonate medium (199 mM NaCl; 4.8 mM KCl; 1.25 mM $MgSO_4$; 25 mM $NaHCO_3$; 1.24 mM NaH_2PO_4; 1.0 mM $CaCl_2$) containing 2 mM HEPES/NaOH (pH 7.4) and 0.3 ug/ml cloramphenicol. The medium was supplemented with glucose (5 mM) and insulin (0.1 U/ml). Muscles were incubated for 30 min at 36°C, then transferred to fresh medium of the same composition and incubated for an additional 3 h. PGE_2 production was determined by radioimmunoassay of the incubation medium as described previously (Tian & Baracos 1989). The cross reactivity of the anti-PGE_2- antibody (ICN Immunobiologicals) with PGE_1 is 150%, but with $PGF_{2\alpha}$ less than 2%. The effective range of the assay was from 12.5-875 pg/sample.

To determine muscle protein content, a separate experiment was carried out. The medial gastrocnemius (MG), soleus (SOL), lateral gastrocnemius (LG) and extensor digitorum longus (EDL) muscles were dissected intact from the injured and injured rats in a second experiment. Muscles were solubilized in 1.0 N NaOH at room temperature and their protein content determined using the Bradford method (Bradford 1976).

C. RESULTS

The Effects of Trauma on Protein Mass of Skeletal Muscle

Medial Gastrocnemius Muscle

The effects of trauma and ultrasound (US) on the protein mass of the medial gastrocnemius muscle were determined (Table 1). Three days post-injury, traumatized muscle showed a significant ($P<0.05$) decrease (-58.0%) in total protein mass when compared to uninjured control muscles. The protein mass of the contralateral muscle that did not undergo direct trauma also was significantly ($P<0.05$) less (-23.0%) than that of the healthy control. Nine days post-injury the protein mass of muscles of all treatments were greater than those on day 3 ($P<0.05$). The protein mass of the traumatized muscle was not significantly different ($P<0.05$) from the trauma control, but both of these were significantly ($P<0.05$) less (-25-28%) than that of the healthy control muscles. On day 9, the protein mass of the trauma and trauma control muscles that received ultrasound therapy were not significantly different ($P<0.05$) from their respective untreated counterparts. However, non-traumatized muscle treated with ultrasound had a significantly ($P<0.05$) lower (-8.7%) protein content than the healthy control at day 9.

Soleus Muscle

On day 3 post-trauma, there was a significant ($P<0.05$) decrease (-30.1%) in the protein mass of the traumatized soleus muscle when compared to the uninjured control muscle (Table 2). The protein mass of the contralateral muscle that did not receive direct trauma was significantly ($P<0.05$) less (-21.7%) than that of uninjured control muscles. On day 9, the protein mass of the trauma, trauma control, and normal control muscles were greater than their counterparts on day 3 ($P<0.05$). The protein mass of the traumatized muscle was significantly ($P<0.05$) less than that of the trauma control (-17.4%) and healthy control (-46.0%) muscles. The protein mass of the trauma control muscle was still significantly ($P<0.05$) less (- 34.6%) than that of the healthy control muscles.

On day 9, the protein mass of the traumatized muscles that underwent ultrasound therapy was significantly ($P<0.05$) greater than that of the untreated traumatized muscles. The trauma control and uninjured control muscles that received ultrasound therapy were not significantly different ($P<0.05$) from their respective untreated counterparts.

Lateral Gastrocnemius Muscle

Three days post-injury, traumatized lateral gastrocnemius muscles showed a significant ($P<0.05$) decrease (-36.5%) in protein mass when compared to the healthy control muscles (Table 3). The protein mass of the contralateral control muscle that did not undergo direct trauma was also significantly ($P<0.05$) less (-13.6%) than that of the healthy control muscles. On day 9, the protein mass of the trauma, trauma control, and uninjured control muscles were greater than their counterparts on day 3 ($P<0.05$). The protein mass of the traumatized muscle was ($P<0.05$) less than the trauma control (-11.3%) and the normal control muscles (-43.5%). Also, the protein mass of the trauma control muscles was still ($P<0.05$) less (-36.3%) than the healthy control muscles. On day 9, in rats subjected to ultrasound therapy from day 3 to day 9, the protein mass of the trauma (+14.8%) and trauma control (+10.9%) muscles were significantly ($P<0.05$) greater than their respective untreated counterparts. However, the protein mass of the healthy control muscles treated with ultrasound was significantly ($P<0.05$) less (-20.2%) than its untreated counterparts.

Extensor Digitorum Longus Muscle

On day 3, in traumatized EDL muscles, there was a significant ($P<0.05$) decrease (-35.4%) in the protein mass when compared to healthy control muscles (Table 4). The protein mass of the contralateral muscles that did not undergo direct trauma was also significantly ($P<0.05$) less (-17.3%) than that of the uninjured control muscles. On day 9, the protein mass of the trauma, trauma control and healthy control muscles were greater than those on day 3 ($P<0.05$). The protein mass of the trauma (-28.0%) and trauma control (-27.2%) muscles were significantly ($P<0.05$) lower than in the healthy control muscles. The protein mass of the trauma control muscles was still significantly less ($P<0.05$) than that of the healthy control muscles. On day 9, in muscles that underwent ultrasound therapy from day 3 to day 9, the protein mass of the traumatized muscle was significantly ($P<0.05$) less (-9.9%) than the trauma control muscles, which was significantly ($P<0.05$) less (-26.3%) than the healthy control muscles. On day 9, the protein mass of the traumatized muscles treated with ultrasound therapy was significantly ($P<0.05$) less (-13.2%) than that of the untreated traumatized muscles. The trauma control and uninjured control muscles treated with US were not significantly different from their untreated counterparts.

Muscle Percent Protein: Effects of Injury and Ultrasound

As seen previously (Chapter III), muscle injury decreased the percent protein seen in all four muscles studied (Tables 5-8). For example, in the injured medial gastrocnemius, percent protein was decreased by (-43%) on day 3 and on day 9 (-30%) ($P<0.05$). The uninjured limb of injured rats also showed a significant drop in percent protein, compared to uninjured controls. This effect was seen in all four muscles studied and was significant ($P<0.05$) but always lesser in magnitude than that seen in the directly injured limb. The effect of ultrasound on muscle percent protein was variable. Ultrasound therapy significantly increased the protein content of injured medial gastrocnemius and soleus, but significantly decreased the protein content of injured lateral gastrocnemius and EDL. Ultrasound therapy for 6 days did not affect the protein content of muscles of healthy control rats.

In a control experiment, rats were given ultrasound treatment as described under Materials and Methods, or sham-treated. At the end of 5 days treatment animals were sacrificed and muscles dissected for determination of protein content and PGE_2 synthesis. Ultrasound therapy had no significant effect on the protein content of the MG, LG, SOL and EDL muscles (data not shown). However, ultrasound therapy decreased the PGE_2 production of the MG muscle from 65.3 ±19.8 pg/mg muscle/3h to 17.3 ±1.19 pg/mg muscle/3 hours incubation in treated muscles ($P<0.05$, $n=6$).

Muscle PGE_2 synthesis was also measured after trauma and ultrasound treatment (Table 9). Observations of PGE_2 release showed considerable variability; the source of this variability is not known. On day 3, in muscles which received trauma, there was a significant ($P<0.05$) increase in PGE_2 production compared to both the trauma control (+99%) and healthy control muscles (+135%). There was no significant difference in PGE_2 production between the trauma control muscles and the healthy control muscles. On day 9, PGE_2 production in the traumatized muscle was still significantly ($P<0.05$) higher than in the trauma control muscle (+255%), and in the healthy control muscles (+203%). On day 9, in muscles that underwent ultrasound therapy, PGE_2 production was significantly different among the ($P<0.05$) trauma, trauma control, and healthy control muscles. On day 9, PGE_2 production in the traumatized muscle undergoing ultrasound therapy was significantly ($P<0.05$) lower (-77.4%) than that in the traumatized muscle which did not receive treatment. Therapeutic ultrasound had no significant effect on the PGE_2 production of the trauma control or healthy muscles.

TABLE 1.

Effects of Trauma and Therapeutic Ultrasound on Muscle Protein Mass

MEDIAL GASTROCNEMIUS MUSCLE

	TRAUMA	TRAUMA CONTROL	CONTROL
		Total Protein Mass (mg)	
DAY 3	12.59 ± 0.59^{c}	23.11 ± 0.70^{b}	30.00 ± 1.34^{a}
DAY 9	33.10 ± 0.67^{b}	34.86 ± 0.22^{b}	46.25 ± 0.71^{a}
Ultrasound DAY 9	33.28 ± 0.57^{b}	36.26 ± 0.72^{b}	$42.22 \pm 0.97^{a*}$

^{a,b,c} Numbers in the same row having different superscripts are significantly different, ($p<0.05$); * indicates a significant ($P<0.05$) effect of ultrasound (n=6).

Rats were given a single impact trauma to the medial aspect of the left lower leg (TRAUMA) or were not injured (CONTROL). Observations of muscle protein content were made on days 3 and 9 after trauma. In traumatized rats, both the injured leg (TRAUMA) and uninjured leg (TRAUMA CONTROL) were studied. Control and injured rats received therapeutic ultrasound or remained untreated. The muscle protein content was determined on the medial gastrocnemius muscle using the Bradford method. Ultrasound treatments were given twice daily starting on day 3 and continuing until day 9 (1.5 watts/cm^2 x 3 min for 6 days) to the medial aspect of the injured hind limb.

TABLE 2.

Effects of Trauma and Therapeutic Ultrasound on Muscle Protein Mass

SOLEUS MUSCLE

	TRAUMA	TRAUMA CONTROL	CONTROL
		Total Protein Mass (mg)	
DAY 3	4.52 ± 0.13^{c}	5.06 ± 0.25^{b}	6.47 ± 0.40^{a}
DAY 9	6.87 ± 0.25^{c}	8.32 ± 0.25^{b}	12.73 ± 0.28^{a}
Ultrasound DAY 9	$8.73 \pm 0.32^{b*}$	9.08 ± 0.25^{b}	11.49 ± 0.61^{a}

[a,b,c] Numbers in the same row having different superscripts are significantly different, ($p<0.05$); * indicates a significant ($P<0.05$) effect of ultrasound (n=6).

Rats were given a single impact trauma to the medial aspect of the left lower leg (TRAUMA) or were not injured (CONTROL). Observations of muscle protein content were made on days 3 and 9 after trauma. In traumatized rats, both the injured leg (TRAUMA) and uninjured leg (TRAUMA CONTROL) were studied. Control and injured rats received therapeutic ultrasound or remained untreated. The muscle protein content was determined on the medial gastrocnemius muscle using the Bradford method. Ultrasound treatments were given twice daily starting on day 3 and continuing until day 9 (1.5 watts/cm^2 x 3 min for 6 days) to the medial aspect of the injured hind limb.

TABLE 3.

Effects of Trauma and Therapeutic Ultrasound on Muscle Protein Mass

LATERAL GASTROCNEMIUS MUSCLE

	TRAUMA	TRAUMA CONTROL	CONTROL
	Total Protein Mass (mg)		
DAY 3	44.77 ± 1.07[c]	61.14 ± 1.51[b]	70.60 ± 2.05[a]
DAY 9	86.33 ± 2.91[c]	97.37 ± 1.08[b]	152.89 ± 1.17[a]
Ultrasound DAY 9	99.13 ± 1.48[c*]	108.48 ± 1.57b*	121.92 ± 0.59[a*]

[a,b,c] Numbers in the same row having different superscripts are significantly different, ($p<0.05$); * indicates a significant ($P<0.05$) effect of ultrasound (n=6).

Rats were given a single impact trauma to the medial aspect of the left lower leg (TRAUMA) or were not injured (CONTROL). Observations of muscle protein content were made on days 3 and 9 after trauma. In traumatized rats, both the injured leg (TRAUMA) and uninjured leg (TRAUMA CONTROL) were studied. Control and injured rats received therapeutic ultrasound or remained untreated. The muscle protein content was determined on the medial gastrocnemius muscle using the Bradford method. Ultrasound treatments were given twice daily starting on day 3 and continuing until day 9 (1.5 watts/cm^2 x 3 min for 6 days) to the medial aspect of the injured hind limb.

TABLE 4.

Effects of Trauma and Therapeutic Ultrasound on Muscle Protein Mass

EXTENSOR DIGITORUM LONGUS MUSCLE

	TRAUMA	TRAUMA CONTROL	CONTROL
		Total Protein Mass (mg)	
DAY 3	7.70 ± 0.29^{c}	9.70 ± 0.25^{b}	11.74 ± 0.55^{a}
DAY 9	11.42 ± 0.42^{b}	11.56 ± 0.23^{b}	15.88 ± 0.36^{a}
Ultrasound DAY 9	$9.91 \pm 0.48^{c*}$	11.01 ± 0.27^{b}	14.94 ± 0.30^{a}

[a,b,c] Numbers in the same row having different superscripts are significantly different, ($p<0.05$); * indicates a significant ($P<0.05$) effect of ultrasound ($n=6$).

Rats were given a single impact trauma to the medial aspect of the left lower leg (TRAUMA) or were not injured (CONTROL). Observations of muscle protein content were made on days 3 and 9 after trauma. In traumatized rats, both the injured leg (TRAUMA) and uninjured leg (TRAUMA CONTROL) were studied. Control and injured rats received therapeutic ultrasound or remained untreated. The muscle protein content was determined on the medial gastrocnemius muscle using the Bradford method. Ultrasound treatments were given twice daily starting on day 3 and continuing until day 9 (1.5 watts/cm^2 x 3 min for 6 days) to the medial aspect of the injured hind limb.

TABLE 5.

Effects of Trauma and Therapeutic Ultrasound on Muscle Percent Protein

MEDIAL GASTROCEMIUS MUSCLE

	TRAUMA	TRAUMA CONTROL	CONTROL
		Percent Protein	
DAY 3	8.65 ± 0.41^{c}	10.92 ± 0.41^{a}	14.61 ± 0.41^{a}
DAY 9	11.95 ± 0.39^{b}	12.17 ± 0.93^{b}	18.52 ± 0.57^{a}
Ultrasound DAY 9	14.14 ± 0.27^{b*}	15.06 ± 0.40^{b*}	18.31 ± 0.60^{a}

[a,b,c] Numbers in the same row having different superscripts are significantly different, $(p<0.05)$; * indicates a significant $(P<0.05)$ effect of ultrasound (n=6).

Rats were given a single impact trauma to the medial aspect of the left lower leg (TRAUMA) or were not injured (CONTROL). Observations of muscle protein content were made on days 3 and 9 after trauma. In traumatized rats, both the injured leg (TRAUMA) and uninjured leg (TRAUMA CONTROL) were studied. Control and injured rats received therapeutic ultrasound or remained untreated. The muscle protein content was determined on the medial gastrocnemius muscle using the Bradford method. Ultrasound treatments were given twice daily starting on day 3 and continuing until day 9 (1.5 watts/cm^2 x 3 min for 6 days) to the medial aspect of the injured hind limb.

TABLE 6.

Effects of Trauma and Therapeutic Ultrasound on Muscle Percent Protein

SOLEUS MUSCLE

	TRAUMA	TRAUMA CONTROL	CONTROL
		Percent Protein	
DAY 3	9.07 ± 0.34^{c}	10.87 ± 0.34^{b}	17.22 ± 1.18^{a}
DAY 9	15.56 ± 1.51^{b}	16.07 ± 1.26^{b}	22.16 ± 1.73^{a}
US DAY 9	17.02 ± 0.66^{b*}	17.80 ± 1.03^{a}	21.63 ± 0.95^{a}

^{a,b,c} Numbers in the same row having different superscripts are significantly different, ($p<0.05$); * indicates a significant ($P\leq0.05$) effect of ultrasound (n=6).

Rats were given a single impact trauma to the medial aspect of the left lower leg (TRAUMA) or were not injured (CONTROL). Observations of muscle protein content were made on days 3 and 9 after trauma. In traumatized rats, both the injured leg (TRAUMA) and uninjured leg (TRAUMA CONTROL) were studied. Control and injured rats received therapeutic ultrasound or remained untreated. The muscle protein content was determined on the medial gastrocnemius muscle using the Bradford method. Ultrasound treatments were given twice daily starting on day 3 and continuing until day 9 (1.5 watts/cm^2 x 3 min for 6 days) to the medial aspect of the injured hind limb.

TABLE 7.

Effects of Trauma and Therapeutic Ultrasound on Muscle Percent Protein

LATERAL GASTROCNEMIUS MUSCLE

	TRAUMA	TRAUMA CONTROL	CONTROL
		Percent Protein	
DAY 3	14.09 ± 0.22^{b}	15.12 ± 0.45^{b}	17.52 ± 0.42^{a}
DAY 9	17.27 ± 0.29^{c}	18.41 ± 0.21^{b}	23.53 ± 0.49^{a}
Ultrasound DAY 9	$15.11 \pm 0.24^{c*}$	$16.63 \pm 0.15^{b*}$	24.21 ± 0.39^{a}

^{a,b,c} Numbers in the same row having different superscripts are significantly different, ($p<0.05$); * indicates a significant ($P<0.05$) effect of ultrasound (n=6).

Rats were given a single impact trauma to the medial aspect of the left lower leg (TRAUMA) or were not injured (CONTROL). Observations of muscle protein content were made on days 3 and 9 after trauma. In traumatized rats, both the injured leg (TRAUMA) and uninjured leg (TRAUMA CONTROL) were studied. Control and injured rats received therapeutic ultrasound or remained untreated. The muscle protein content was determined on the medial gastrocnemius muscle using the Bradford method. Ultrasound treatments were given twice daily starting on day 3 and continuing until day 9 (1.5 watts/cm^2 x 3 min for 6 days) to the medial aspect of the injured hind limb.

TABLE 8.

Effects of Trauma and Therapeutic Ultrasound on Muscle Percent Protein

EXTENSOR DIGITORUM LONGUS MUSCLE

	TRAUMA	TRAUMA CONTROL	CONTROL
		Percent Protein	
DAY 3	13.44 ± 1.09^{b}	16.75 ± 0.81^{b}	20.51 ± 1.27^{a}
DAY 9	16.30 ± 0.58^{b}	18.15 ± 1.29^{b}	23.27 ± 1.16^{a}
Ultrasound DAY 9	14.66 ± 0.89^{c*}	16.58 ± 0.74^{b}	22.27 ± 0.60^{a}

^{a,b,c} Numbers in the same row having different superscripts are significantly different, ($p<0.05$); * indicates a significant ($P<0.05$) effect of ultrasound (n=6).

Rats were given a single impact trauma to the medial aspect of the left lower leg (TRAUMA) or were not injured (CONTROL). Observations of muscle protein content were made on days 3 and 9 after trauma. In traumatized rats, both the injured leg (TRAUMA) and uninjured leg (TRAUMA CONTROL) were studied. Control and injured rats received therapeutic ultrasound or remained untreated. The muscle protein content was determined on the medial gastrocnemius muscle using the Bradford method. Ultrasound treatments were given twice daily starting on day 3 and continuing until day 9 (1.5 watts/cm^2 x 3 min for 6 days) to the medial aspect of the injured hind limb.

TABLE 9.

The Effects of Trauma and Therapeutic Ultrasound on PGE_2 Production

MEDIAL GASTROCEMIUS MUSCLE

	TRAUMA	TRAUMA CONTROL	CONTROL
		pg PGE_2/mg muscle/3 h	
DAY 3	206.6 ± 82.2^{b}	103.7 ± 37.8^{a}	88.1 ± 37.8^{a}
DAY 9	222.0 ± 108^{b}	62.5 ± 10.5^{a}	73.2 ± 29.2^{a}
Ultrasound DAY 9	$50.1 \pm 12.3^{a*}$	65.5 ± 3.9^{b}	42.8 ± 14.0^{a}

[a,b] Numbers in the same row having different superscripts are different, ($p<0.05$); [*] Significant effects of ultrasound, ($p<0.05$) ($n=6$).

Rats were given a single impact trauma to the medial aspect of the left lower leg (TRAUMA) or were not injured (CONTROL). Observations of PGE_2 synthesis were made on days 3 and 9 after trauma. In traumatized rats, both the injured leg (TRAUMA) and uninjured leg (TRAUMA CONTROL) were studied. Control and injured rats received therapeutic ultrasound or remained untreated. Ultrasound treatments were given to the medial aspect of the injured hind limb twice daily starting on day 3 and continuing until day 9. At the time points indicated, the medial gastrocnemius muscles were dissected and a small biopsy incubated. Production of PGE_2 was measured by the release of PGE_2 into the incubation medium.

D. Discussion

We have undertaken to study the effects of therapeutic ultrasound on skeletal muscle following acute blunt trauma. The experimental injury device offers a simple and humane experimental method of producing acute blunt trauma to skeletal muscle. This protocol was developed from one used previously by Stratton et al.(1984) for evaluation of therapeutic ultrasound, and was intended to produce an injury approximating a muscle contusion of moderate severity. The reproduceability of the injury was verified by determining the tissue protein content, which was highly consistant within groups and time points. The results of these experiments on protein content agree with our previous studies following muscle injury and repair (Chapter II, III). During the first three days following acute blunt trauma there was a marked reduction in protein content of muscles at the site of injury and this was followed by repletion over the the next six days which, however, does not reach that of control values over this time interval.

Ultrasound had variable effects on the protein mass and percent protein of the muscles studied. Ultrasound increased the protein mass of injured SOL and LG, did not affect MG and had an adverse effect on EDL. Ultrasound tended to decrease the protein mass of healthy control muscles: for MG and LG this effect was significant.

Alterations in muscle protein content during the acute inflammatory response appear to depend partly on arachidonic acid metabolism and the production of prostaglandins (Chapter III), which are acute inflammatory mediators. The work of several authors indicates that PGE_2 and PGF_2 signal changes in muscle protein turnover (Rodemann & Goldberg 1982, Reeds & Palmer 1984, Baracos et al. 983, Tian & Baracos 1989). PGE_2 increased protein degradation (without affecting protein synthesis); whereas, PGF_{2alpha} stimulated protein synthesis without affecting protein degradation (Rodemann and Goldberg 1982).

In our study, therapeutic ultrasound produced a significant reduction ($P>0.03$) in the production of PGE_2 of healthy control gastrocnemius muscles when compared to untreated muscles. An additional study was conducted to determine the effect of therapeutic ultrasound on PGE_2 synthesis in traumatized muscle. PGE_2 production was observed at day 3 and day 9. The results from this study were inconclusive due to the large standard errors, however, there was a significant decrease in PGE_2 production in the muscles which received ultrasound therapy when compared to traumatized non-treated muscles. In contrast to our results, the work of Horrobin (1978) and Baen Zigeret al. (1977) have indicated that ultrasound may trigger the release of prostaglandins from the capillary epithelium. At this point it is too early to provide firm data and further studies need to be carried out to quantify and identify the prostaglandins released following the application of therapeutic ultrasound to skeletal muscle.

At the present time, an explanation of the effects of ultrasound on muscle protein mass seen in this study, remains a matter for speculation. Many factors might influence the muscle response to ultrasound, such as the degree of trauma, and the regenerative capacity of the muscle. Ultrasound may be influencing the PGE_2 production of the muscle tissue and hence its protein metabolism, however, other inflammatory mediators may also be affected. The effect of the ultrasound treatment could vary according to the dose and the time of application relative to the injury.

The efficacy of a therapy may be evaluated by a variety of parameters. Histological criteria have been used previously to evaluate ultrasound therapy (Stratton et al. 1984). Criteria such as muscle protein accumulation (Chapter III), the concentration of cellular metabolites, mediators (Fyfe & Chahl 1984), tissue strength (Dyson & Suckling 1978) and composition (Griffin 1966) have also been used. In the present study we have used the overall replacement of tissue protein over a fixed time period as a measure of healing rate in treated and untreated subjects. This parameter may be accurately quantified, but does not indicate the composition of the protein replaced. It would appear appropriate to determine whether the functional capacity of muscles was affected by ultrasound therapy, as judged by other criteria.

In the present study, therapeutic ultrasound was effective or not effective in promoting muscle protein repletion. In one case, ultrasound therapy significantly impaired protein repletion. Thus our results would appear to concur with those of others (e.g. Goddard 1983) that ultrasound is not necessarily an effective treatment. It must be considered that only one treatment protocol was used in terms of dose, duration, and time frame of ultrasound application relative to the injury. Further work is required to demonstrate if any of these influence net protein repletion or other functional characteristics after muscle injury.

REFERENCES

1. Arnheim, D. 1989. Modern principles of athletic training. Time Mirror/Mosby College Publishing St. Louis, Toronto pp. 339-366

2. Baracos V., Rodemann H.P., Dinarello C.A., Goldberg A.L. 1983. Stimulation of muscle degradation and prostaglandin E2 released by leukocytic pyrogen (interleukin-1) N. Eng. J. Med. 309:553

3. Baen Ziger N.L., Dillender M.J., Majerus P.W. 1977. Cultured human skin fibroblast and arterial cells produce a labile platelet inhibitory prostaglandin. Biochem Biophys. Res. Comm. 78:294-301

4. Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein dye binding. Anal. Biochem. 72:248-254

5. Davis J., Shires T. 1986 Host Defense in trauma and surgery. Raven Press, New York. 111-142

6. Dyson M., Suckling J. 1978. Stimulation of tissue repair by ultrasound: A survey of the mechanism involved. Physiotherapy 64: 105-108

7. Fyfe M., Chahl L. 1980. The effects of ultrasound on experimental oedema in rats. Ultrasound Med. Biol. 6:107-111

8. Fyfe M., Chahl L. 1984. Mast cell degranulation and increased vascular permeability induced by therapeutic ultrasound in the rat ankle joint Br. J. Exp. Path. 65:671-676

9. Goddard D.H., Pevell P.A., Carson J. 1983. Ultrasound has no anti-inflammatory effect. Ann. of the Rheu. Dis.: 42, 582-584

10. Griffin J. 1966. Physiological effects of ultrasonic energy as it is used clinically. J. Amer. Physical Ther. Assoc.: 46, 1, 18-26

11. Harvey W., Duson M., Pond J. 1975. The stimulation of protein synthesis in human fibroblast by therapeutic ultrasound Rheumatal Rehabil. 13:237

12. Horrobin D.F. 1978. Prostaglandins: Physiology, Pharmacology and Clinical Signifiance. Edinburgh: Churchill Livingstone 17-18

13. Lehmann J., DeLateur B., Stonebridge J. 1967. Therapeutic temperature distribution produced by ultrasound as modified by dosage and volume of tissue exposed. Arch. Phys. Med. 48:662-666

14. Paul B.J., La Pratta C.W., Dawson A.R., Baar E., and Bullock P. 1960. Use of ultrasound in the treatment of pressure sores in patients with spinal cord injury. Arch. Phys. Med. & Rehab. 41:438-441

15. Reeds P., Palmer R. 1984. The possible involvement of prostaglandin F2α in the stimulation of muscle protein synthesis by insulin. Biochem. Biophys. Res. Commun. 3:1084-1094

16. Reid D.C., 1981. Contraindications and Precautions Associated with Ultrasound Therapy, in An International Symposium on Therapeutic Ultrasound Proc. Winnipeg, Canada Sept 10-12, 273-290

17. Rodemann H.P., Goldberg A.L. 1982. Arachidonic acid, prostaglandin E_2 and $F_{2\alpha}$ influence rates of protein turnover in skeletal and cardiac muscle. J. Biol. Chem. 257(4):1632-1638

18. Reilly T. 1981. Sport Fitness and Sport Injuries. Faber and Faber London & Boston 259-265

19. Stratton S., Heckmann R., Rulon F. 1984. Therapeutic ultrasound: Its effects on the integrity of a nonpenetrating wound. J. Orthopaedic Sport Physio. Ther.: Mar./Apr., 278-281

20. Tian S., Baracos V.E. 1989. Prostaglandin-dependent muscle wasting during infection. Physiotherapy 64:105-108

CHAPTER V: GENERAL DISCUSSION AND CONCLUSIONS

Muscle injuries are common to sport and with a greater number of participants and greater competitive standards and intensity at all levels of sport, the need for more effective care has become essential. The requirements and demands of practice sessions and competition make it difficult for athletes to compete with even minor injuries. For competitive athletics, these injuries represent a major problem, for they usually prevent the athlete from effective performance. If injuries are mismanaged they can become chronic and are extremely difficult to treat successfully. Early recognition of the muscle injury is critical. Even though these injuries do not cause serious problems in everyday life, the care givers must understand the significance of physical activity to athletes and to the population as a whole and treat them effectively.

The following conclusions can be drawn from this thesis:

1. The protein mass of the injured muscles was significantly reduced when compared to the contralateral muscles as well as those from healthy rats for the first three days following trauma. Protein loss was also observed in uninjured limbs of traumatized rats, compared to healthy controls.

2. When skeletal muscle is traumatized the muscle undergoes a

degenerative process followed by a regenerative process. The degenerative response is characterized by acute inflammatory mediators and a catabolic response. The degenerative process lasts for approximately 3 days following trauma. The regenerative phase begins around day 3 and continues until day 21. This phase is characterized by the presence of satellite cells, myoblasts, the presence of myofibrils forming sarcomeres, and undifferentiated muscle fibres.

3. The administration of naproxen appeared to inhibit the acute catabolic loss of muscle protein and enhanced the rate of tissue protein repletion, in both limbs of the traumatized rat.

4. Ultrasound had no effect on the traumatized medial gastrocnemius muscle (the muscle which received the direct impact from the injury device). Protein repletion in the injured soleus and lateral gastrocnemius muscles was enhanced following insonication. By contrast, ultrasound had an inhibitory effect on the regrowth of the extensor digitorum longus following trauma. These variable results with different muscle groups require further investigation.

A number of related points for discussion arise from this thesis.

I. What constitutes an effective therapy?

Therapy for muscle injury may be divided into several components: 1) relief of symptoms, particularly pain, 2) prevention of further tissue damage, 3) restoration of function in the shortest time period by a) decreasing the acute inflammatory response, b) promoting the regeneration of contractile protein in muscle, c) returning the muscle to its original functional capacity, and d) decreasing the formation of collagenous (scar) tissue.

To relieve the symptoms associated with an acute muscle injury the treatment should provide rest, avoidance of weight bearing, ice, compression, and elevation. The initial rest period should continue for a period of time to prevent any further damage to the sarcolemma and to allow the synthesis of new collagen to areas which were damaged during the initial injury. If return to activity is premature the sarcolemma may be compromised allowing the infiltration of fibroblasts within the myofibre; this may increase the likelihood of scar formation.

To decrease the acute inflammatory response the application of ice promotes vasoconstriction, increases blood viscosity, decreases cell metabolism, and decreases the neuroimpulses which trigger the pain response. The administration of NSAIDs immediately following injury will reduce the acute inflammatory mediators which produce pain and add further to the catabolic response.

The regenerative process also requires the production of the appropriate myofibrillar proteins. These proteins are products of a highly complex multigene group that undergo a developmentally and temporally controlled sequence of replacement of individual isoforms (Woo 1987). Future research should consider this component of the regenerative process in an attempt to understand what is involved in the regenerative response.

Following the initial rest period careful but active treatment involving early mobilization in mild muscle contractions can be carried out. Renstrom (1988) reported that healing of partial muscle strain in skeletal muscle was accelerated by early mobilization treatment when compared to immobilization. Letho et al. (1985) also reported that immobilization following injury accelerated the formation of scar tissue and poor realignment of regenerating muscle in the scar tissue component. However, Renstrom (1988) stated that an immobilization period of about five days was required in the injured rat muscle to allow newly forming connective tissue to cover the entire injury site. This also allowed the sarcolemma sufficient time to gain tensile strength to resist the forces produced by early mobilization treatment.

Exercise is another important factor in an effective rehabilitation program. Therapeutic exercise should be focused toward returning the muscle to its original functional capacity and decreasing the formation of collagenous (scar) tissue. This will preserve the elasticity of the sarcolemma and maintain the strength and contractility of the muscle unit (Ciullo & Zarins 1983). The exercise program requires careful implementation with a gradual increase of the load and exercise which is below the pain threshold.

Once the athlete is pain free, active range of motion exercise involving the injured muscle and gradual increases in load can be applied. However, Arnheim (1989) reported that strength training alone has a negative effect on muscle flexibility and this should be counteracted with stretching programmes. Various therapeutic modalities include whirlpool, contrast bath, interferential current, laser and ultrasound. These therapeutic modalities are all reported to produce a heating effect and relieve some of the symptoms of inflammation (Fyfe & Chahl 1984, Griffen 1962, Arnheim 1989). However, there is little scientific evidence that these modalities will shorten the healing time.

Muscle injury constitutes a diagnostic and therapeutic problem and the symptoms are often diffuse and uncharacteristic. The scientific knowledge regarding muscle injury has increased, yet, treatment is based on practical/empirical experience. More research is required to determine the most effective way to rehabilitate the injured muscle.

II. By what criteria may the degenerative and regenerative events following trauma be experimentally measured?

In this thesis, two assessment criteria were used. The ultrastructural changes in the injured muscles were observed. This method allowed determination and classification of the progressive events at the injured site, with a high degree of resolution. The inherent limitation of ultrastructural studies is the limited field area of observation, and thus the results are more qualitative than quantitative. In addition, it is not possible to determine the identity of cells without the use of specific stain or marker, and the origin of new cells appearing within the injured site cannot be identified. For example Stauber et al.(1988) have provided evidence regarding the nature of cellular infiltrates after forced lengthening of muscle. Here the authors offered evidence that the infiltrating cells observed in the injured muscle were myofibre in origin; they were responsible for the reestablishment of the myofibre following injury.

In the present study, muscle degeneration and regeneration were also assessed by measurement of total protein content according to the method of Bradford (2). The Bradford method involves protein quantitation by binding of coomassie brilliant blue to protein. Some differences in dye binding have been noted for different proteins (Kley & Hale 1977) with this method; alternative methods such as that of Lowry (1951) also have this problem.

Measurements of total protein provide a gross description of the catabolic and subsequent anabolic events after injury. At the same time, there are many classes of proteins as well as individual proteins that would merit study. These might include structural and contractile proteins of the myofibril, cell membrane, and connective tissue. For example, individual components of the connective tissue such as sulfated and unsulfated chondroitin proteoglycan and laminin have been studied in muscle injury (W.T. Stauber, personal communication). Unsulfated proteoglycan is synthesized by prefusion myoblasts (Hutchison & Yasin 1986) while sulfated proteoglycan is synthesized after fusion; thus the presence of these proteoglycans relates to the stage of differentiation of the myogenic cell population. Laminin is a major constituent of the basement membrane (Woo 1987) and may be used as an index of the reformation of this structure.

Many specific enzymatic activities would be of interest to measure in injured muscle. In particular, hydrolytic enzymes responsible for the degradation of muscle protein have been studied (Kettlehut 1988). Muscle cells are known to contain several distinctive proteolytic systems. One of these resides in the lysosome, wherein a large number of acid proteases (cathepsins) as well as other acid hydrolases can act on both membrane and soluble cytosolic proteins. The lysosomal system becomes activated following injury and during starvation (Rennie 1985). Its contribution to overall proteolysis in normal muscle is much less significant. Muscle also exhibits considerable proteolytic activity (extra-lysosomal) at neutral pH. One of these is the Ca^{+2} -dependent protease (Furuno & Goldberg 1986). However, the bulk of proteins comprising the myofibillar apparatus (e.g., actin) are not degraded by either the lysosome or the Ca^{+2} -dependent pathway in the muscle (Ciechanover 1987). Muscle cells also possess an energy dependant proteolytic pathway which is fueled by ATP (Etlinger and Goldberg 1977). When ATP in muscle is depleted in vitro in a Ca^{+2} -free medium, the breakdown of muscle proteins, including actin, falls by 50-70 % (Goldberg & Dice 1974). Another class of enzymes of interest would be those enzymes participating in energy metabolism. These must adapt to the energy demands of injured and regenerating muscle, frequently under ischemic conditions. The activity of these enzymes must also be adequate to support contractile activity in healed muscle.

III. How might these results be applied to the human athlete?

The various therapies used here (ultrasound, NSAID) have been used empirically with benefit in clinical sports medicine. However, optimal times and doses have not been standardized. The experimental model developed, herein, provides an objective means for the quantitative assessment of the therapeutic modalities which have been used clinically, with possible new insights concerning optimal times for the initiation and termination of treatment and mechanisms of action. However, species differences do not permit a direct application of results obtained in the rat to the clinical situation in man.

The experimental results reveal for the first time that there are differences in response, particularly to ultrasound in different muscle groups, which are likely related to differences in function and metabolism.

The ultrastructural and biochemnical findings reported herein indicate that the healing process in muscle extends beyond the duration of the symptoms caused by injury. Hence, the time of return to sports activity should be determined by the former, rather than the latter.

In the present study, the early administration of NSAID post-injury, diminished protein catabolism in the damaged muscle, and accelerated the rate of protein repletion. In sports medicine, it has been clinical practice to delay the administration of NSAID until 24 hours post-injury. Earlier administration of NSAID may be of benefit in sports medicine, but possible gastro-intestinal side-effects and possibly increased bleeding time have to be taken into consideration as well.

In the rat model employed, post-injury protein catabolism occured both in the injured muscle and in the contra-lateral homologous muscle. This peripheral muscle wasting varied with muscle type, and could be of suffucient magnitude to influence athletic performamnce. It would seem important to apply treatment(s) to limit this systemic effect as well as the local reponse in the injured limb.

IV. What are the factors affecting protein metabolism in the injured site?

In the present study we have obtained evidence that prostaglandins might be important in regulation of catabolic events in muscle following injury. These results agree with those of Tian and Baracos (1989) who showed that PGE_2 may be important in muscle wasting associated with systemic infection. These results also support the conclusion of Streklov et al. (1989) who suggested that PGE_2 produced by a malignant tumour may induce systemic muscle wasting. The present results do not agree with those of May et al. (1986) for muscle protein degradation associated with acidosis, or those of Clark et al. (1984) for burn injury. Thus it appears that PGE_2 may not be involved in muscle wasting associated with all illnesses.

A number of systemic and regional factors may also contribute to protein depletion from muscle following trauma: (a) Adrenal glucocorticoids released in the acute stress reaction following trauma stimulate protein degradation in muscle by increasing proteolysis and inhibiting protein synthesis (Tischler & Fagan 1983). The accelerated degradation of myofibrillar proteins induced by glucocorticoids in particular seems to involve the ATP-dependent proteolytic pathway (Furuno & Goldberg 1986). (b) Cytokines: There is now evidence that systemic host reponses to trauma and infection are initiated by circulating factors released by macrophages upon interaction with damaged tissue, bacterial or viral pathogens or immune complexes (Baracos et al. 1983). The best studied of these cytokines are interleukin-1 and tumor necrosis factor (TNF), which are reported to have an overall catabolic effect on muscle proteins (Nathan 1987).

Interleukin-1 also causes a large increase in PGE_2 in muscle, which in turn augments protein catabolism. Although TNF can induce fever, it does not appear to directly increase proteolysis in muscle (Kettlehut et al. 1988). Other products of activated macrophages that are responsible for a negative protein balance in muscle still remain to be identified.

In normal rat muscle, the effect of insulin is to conserve protein by decreasing proteolysis and increasing protein synthesis and glucose utilization. In traumatized rat soleus muscle, protein degradation which is inhibitable by insulin, is diminished, while stimulation of protein synthesisby insulin is unaffected. Thus, in trauma, there is an insensitivity of proteolysis to insulin (Tischler & Fagan, 1983).

V. What are the inherent advantages and limitations of the injury model?

In the model used here, trauma was administered to anaesthetized animals. With halothane anaesthesia producing some muscle relaxation (due to the anaesthesia) the damaging effects of a single impact trauma could be less than in a fully conscious animal because the flacid muscle belly may be displaced more readily, and therefore may absorb less of the impact energy. Inasmuch as the animal is not conscious at the time of injury, the degree of acute neurogenic shock is likely less. Humane considerations preclude injury of conscious animals.

The device used in this study produces an injury approximating the clincial lesion of a muscle contusion of moderate severity. The results of our 31 day study ultrastrastructural study showed that following injury, the muscle was capable of regeneration. In parallel to the ultrastructural events noted, the injured muscle underwent a marked catabolic response, and showed a reproducible fall (-35% P>0.05) in the total protein content within 24 hours. Muscle protein accumulation commenced after day 3, and a complete repletion occured by day 21. The individual variation of tissue protein content on any day was not greater than 5 to 8 % of the mean value.

Different models of muscle injury produce somewhat different patterns of damage to muscle cells, stroma, vessels and nerves, i.e. focal versus pathchy versus diffuse injury; degree of damage to sarcolemmal sheath; presence or absence of interstitial hemmorhage. Therefore different patterns of inflammatory response occur in different models and there can be different time frames for recovery. Because of species differences, including differences in body temperature, metabolic rates, etc, in the time-course, sequences of injury/inflammation/repair cannot be directly extrapolated from the rat to man.

In sports, muscle injury can be the result of energy depletion, mechanical strain or direct injury. The model system employed in the present study is specifically directed to a better understanding of the latter form of injury with regard to the time course of events in the sequence of injury/inflammation/repair, on the basis of ultrastructural findings, protein depletion and repletion, and the effects of therapeutic modalities of ultrasound and NSAID on the foregoing.

We modified the injury device to produce a mild to moderately severe injury that corresponded to similar injuries encountered in sports medicine. In the present study, because of the focal nature of the impact injury to the gastrocnemius muscle in one limb it was feasible to study and compare a) the local effects in the traumatized gastrocnemius muscle, with b) secondary effects on adjacent muscles in the same limb, with c) the systemic effects in the homologous muscles in the contralateral (uninjured) limb. Such comparisons have not been reported in other studies (McKinley & Turinsky 1986) in which there was unilateral muscle injury, and indeed are not possible in model systems in which there was bilateral muscle injury.

REFERENCES

1. Arnheim, D. 1989. Modern Principles of Athletic Training. Time Mirror/Mosby Publishing Co., St.Louis. pp.339-366.

2. Baracos, V.E., Rodemann, H.P., Dinarello, C.A., & Goldberg, A.L. 1983. Stimulation of muscle protein degradation and prostaglandin E_2 release by leukocyte pyrogen (interleukin-1). Fed.Proc. 43:1483-1486.

3. Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248-254.

4. Chiang, H.L., Dice, J.F., 1988. Peptide sequences that target protein for enhanced degradation during serum withdrawal. J. Biol. Chem. 263:6796-6805.

5. Ciechanover, A. 1987. Mechanism of energy-dependent intracellular protein breakdown. The ubiquitin mediated proteolytic pathway. In Lysosomes: Their role in protein breakdown. Academic Press, London. pp.561-602.

6. Ciullo, J.V., Zarins, B., 1983. Biomechanics of the musculotendinous unit. Relation to athletic performance and injury. Clin. Sports Med. 2:71.

7. Clark, J.S., Kelly, R.A., Mitch, W.E. 1984. Systemic response to thermal injury in rats. J. Clin. Invest. 74:888-897.

8. Etlinger J., & Goldberg A.L. 1977. ATP-dependent proteolytic system responsible for the degradation of abnormal protein in reticulocytes. Proc. Natl. Acad. Sci. USA, 74:54-58.

9. Florini, J.R. 1987. Hormonal control of muscle growth muscle. Nerve. 10:577-598.

10. Furuno, K. and Goldberg, A.L. 1986. The activation of protein degradation in muscle by calcium or muscle injury does not involve a lysosolmal mechanism. Biochem. J. 237:859-864.

11. Fyfe, M.CF., Chahl, L.S. 1984. Mast cell degranulation and increased vascular permeability induced by therapeutic ultrasound. Br. J. Exp. Path. 66:671-676.

12. Goldberg, A.L., Dice J.F. 1974. Intracellular protein degradation in mammalian cells. Annu.Rev. Biochem. 43:835-864.

13. Goldberg, A.L., St. John, A.C. 1976. Intracellular protein degradation in mammalian and bacteria cells. Part 2. Annu.Rev.Biochem. 45:747-803.

14. Griffin, J.E., 1966. Physiological effects of ultrasonic energy as it is used clinically. J. Amer. Physio. Ther. Assoc. 46:18-26.

15. Hutchison, C.J., Yasin, C.J. 1986. Developmental changes in sulphation of chondroitin sulphate proteoglycans during myogenesis of human muscle culture. Div. Biol. 115:78-83.

16. Kettlehut, I.C., Wing, S.S., Goldberg, A.L. 1988. Endocrine regulation of protein breakdown in skeletal muscle. Diabet. Meta. Rev. 4:751-772.

17. Lowry, E., Roseborough, N.J., Farris, A. 1951. Protein measurement. Br. J. Nutr. 27:597-100.

18. Letho, M., Sim. T.J., Bailey, A.J. 1985. Skeletal muscle injury, molecular changes in the collagen during healing. Res Exp. Med. 185:95-106.

19. May, R.C., Kelly, R.A., & Mitch, W.E. 1986. Metabolic acidosis stimulates protein degradation in rat muscle by a glucocorticoid-dependent mechanism. J. Clin Invest. 77:614-621.

20. Nathan, C.F. 1987. Secretory products of macrophages. J. Clin. Invest. 79:319-326.

21. Rennie, M.J. Muscle protein turnover and the wasting due to injury and disease. Brit. Med. Bull. 41:257-264.

22. Renstrom, P. 1988. The Olympic Book of Sports Medicine. Blackwell Scientific Publication. London 1. pp.413-427.

23. Stauber W.T., Fritz, V.K., Vogelbach, D.W., Dahmann, B. 1988. Characterization of muscle injured by forced lengthening. I. Cellular infiltrates. Med. Sci. Sport Exer. 20:345-353.

24. Strelkov, A.B., Fields, A.L., Baracos, V.E. 1989. Effects of systemic inhibition of prostaglandin production on protein metabolism in tumor-bearing rats. Amer. J. Physiol. 257:C261-269.

25. Tian, S., Baracos, V.E. Prostaglandin-dependent muscle wasting during infection in broiler chick (Gallus domesticus) and the laboratory rat (ratus norvegicus). Biochem. J. 263:485-490.

26. Tischler M.E., Fagan, J.M. 1983. Response to trauma of protein, amino acid, and carbohydrate metabolism in injured and uninjured rat skeletal muscle. Metabol. 32:853-860.

27. Van Kley, H., Hale, S.M. 1977. Assay for protein by dye binding. Anal. Biochem. 81:485-487.

24. Woo, S., Buckwater, J.A. 1987. Injury and Repair of Musculoskeletal Soft Tissue. American Academy of Orthopaedic Surgeons. Illinois. pp.209-212.

PLATE # 1 IMMEDIATELY POST-TRAUMA

Low power electron micrograph to show mechanical disruption of a muscle fibre with extrusion of sarcoplasmic debris.

PLATE # 2 SIX HOURS POST-TRAUMA

Interstitial hemorrhage due to rupture of a capillary between muscle fibres.

169A

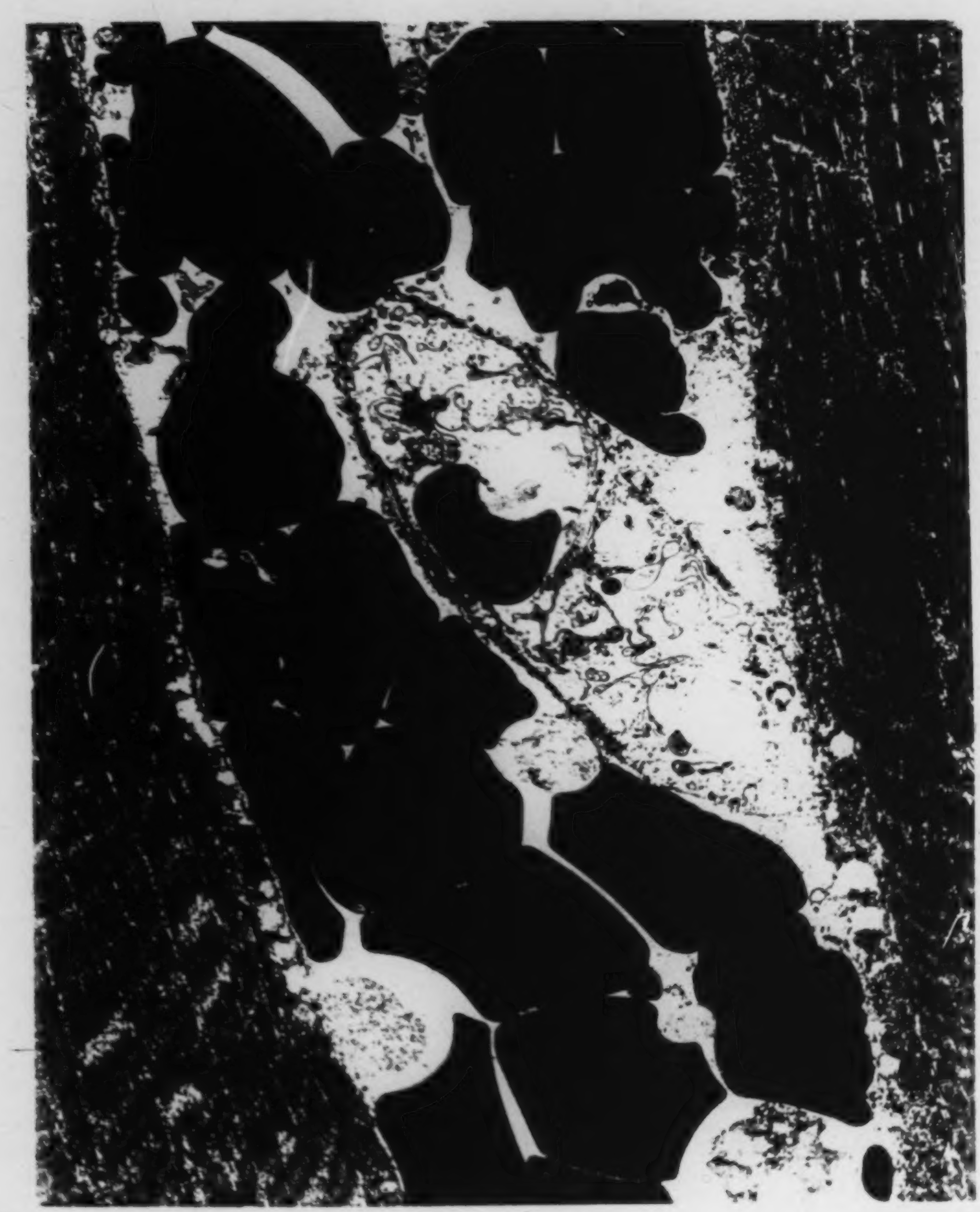


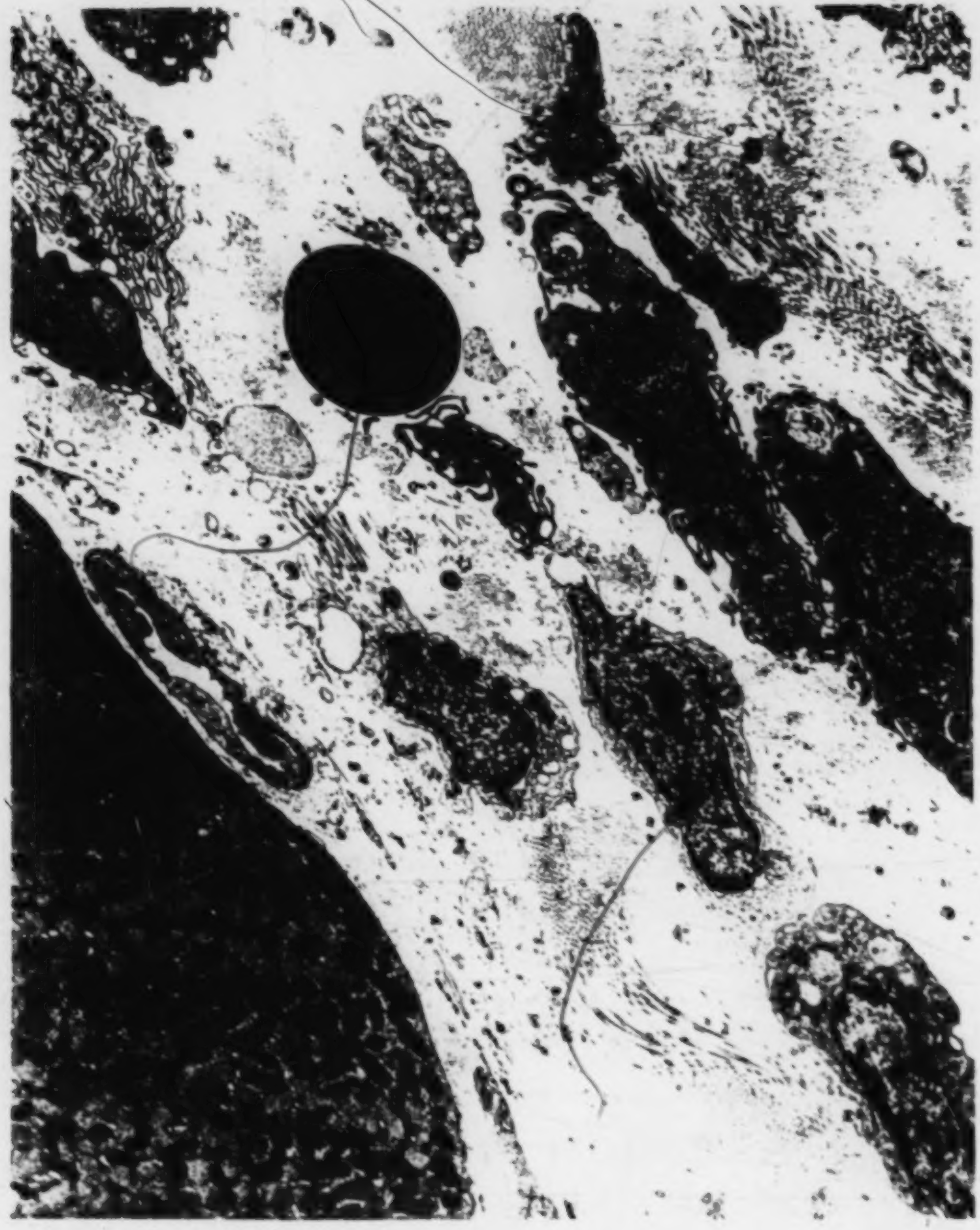
PLATE # 3 SIX HOURS POST-TRAUMA

The endomysium is edematous and is infiltrated by activated macrophages. An activated fibroblast with abundant rough endoplasmic reticulum is present in the upper left hand corner of the field. collagen fibres are present in normal numbers.

PLATE # 4 ONE DAY POST-TRAUMA

A macrophage is present in the exudate between three muscle fibres. At the bottom of the micrograph there is a degenerating muscle fibre whose plasma membrane has disappeared but whose basement membrane persists. Underlying myofibrils are broken and separated from one another.

170A



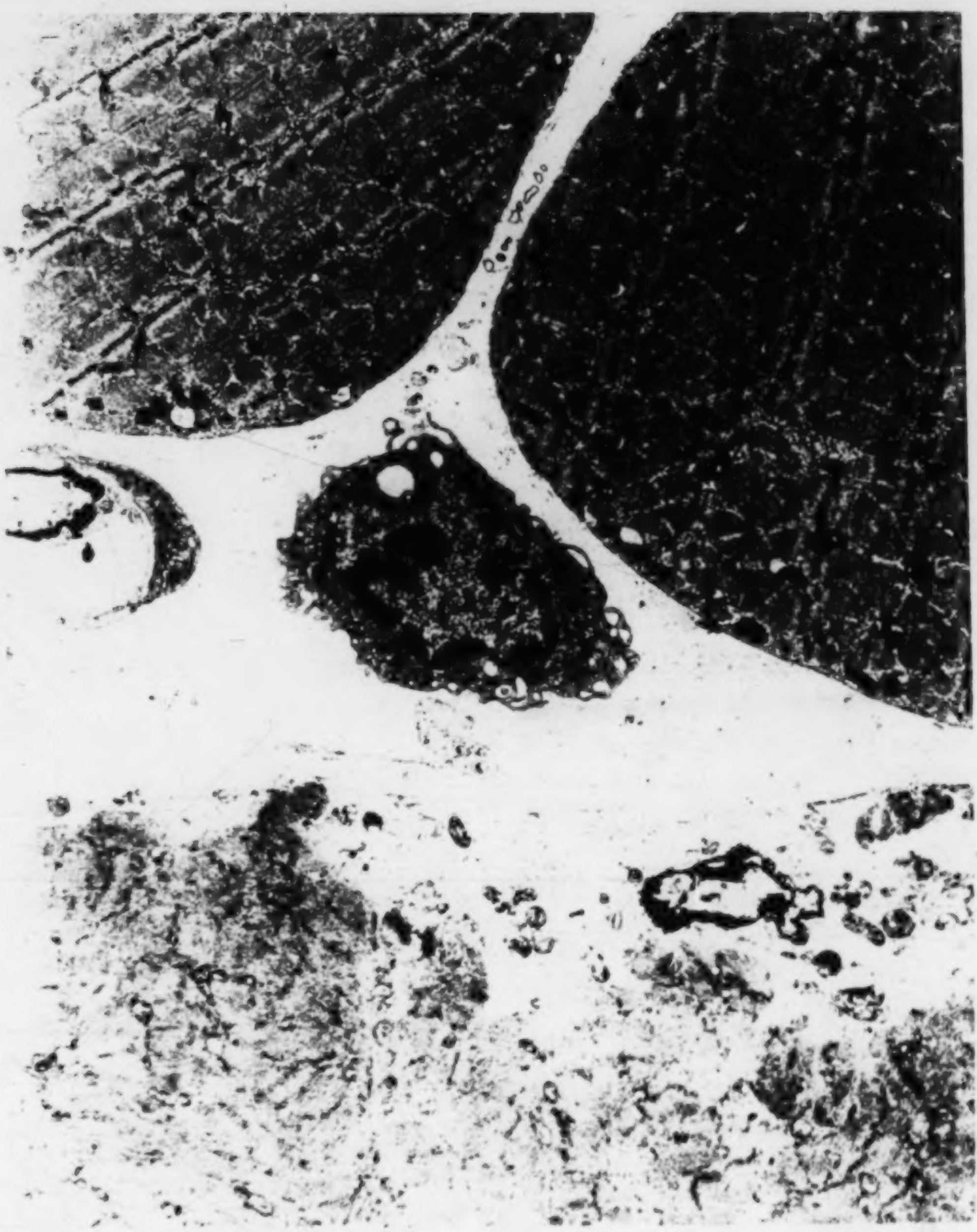


PLATE # 5 ONE DAY POST-TRAUMA

Extensive invasion of a severely injured muscle fibre by macrophages. Invading macrophages contain numerous lysosomes, mitochondria, cisternae of rough endoplasmic reticulum and Golgi Apparatus.

172A

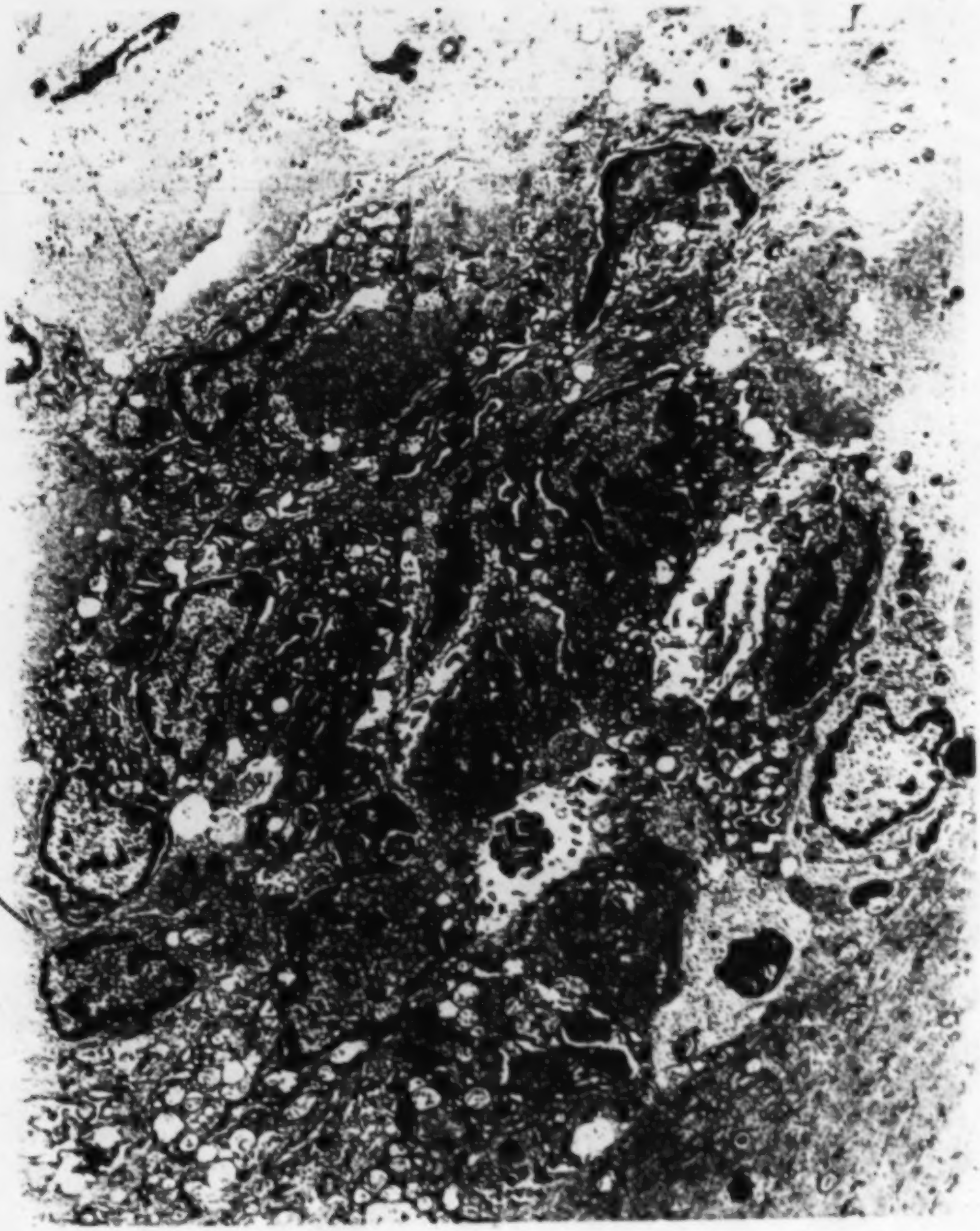


PLATE # 6 ONE DAY POST-TRAUMA

Macrophages have entered a severely damaged muscle fibre whose basement is still intact in the plane of section. The outer zone of the muscle fibre appears normal and is surrounded by collagen fibres of the endomysium.

173A

PLATE # 7 ONE DAY POST-TRAUMA

Extensive invasion of the endomysial stroma by activated macrophages. These cells have a ruffled cytoplasmic border and are rich in primary and secondary lysosomes.

174A

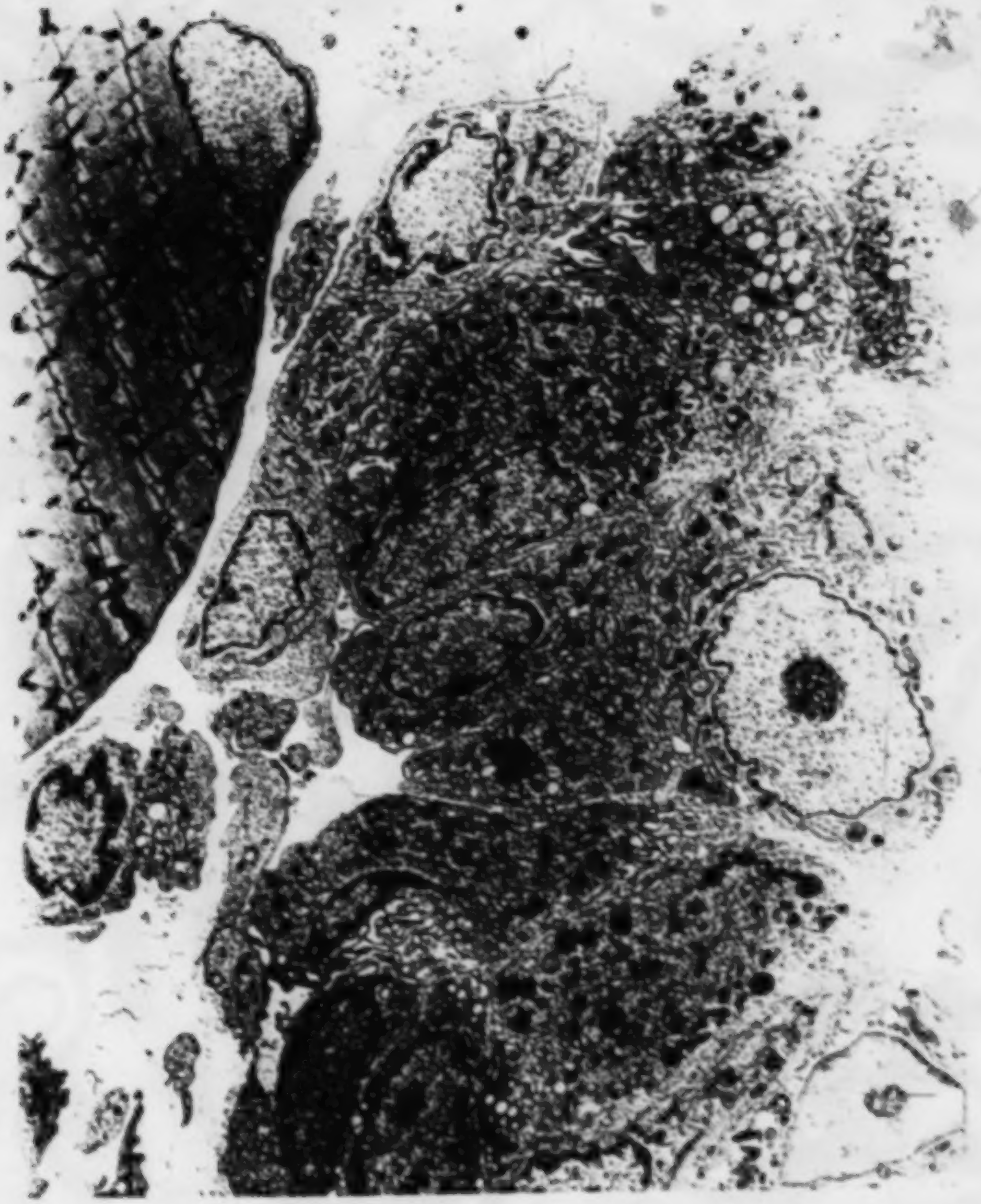


PLATE # 8 TWO DAYS POST-TRAUMA

This reversibly damaged muscle fibre contains large membrane-bound vacuoles, which probably are derived from the sarco-tubular reticulum. Myeloid figures with partially degraded organelles are present in the lower half of the micrograph.

175A

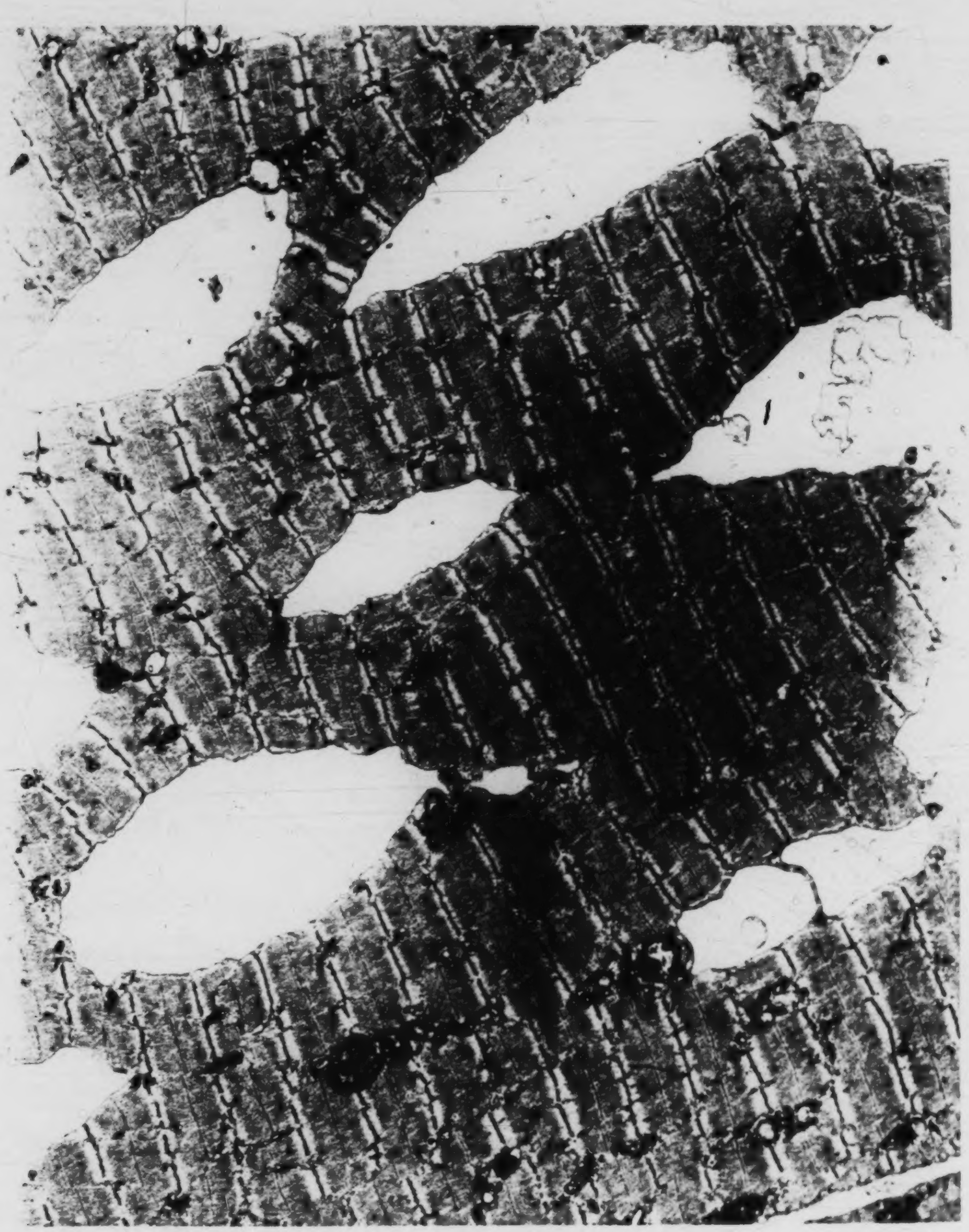


PLATE # 9 TWO DAYS POST-TRAUMA

A regenerating muscle fibre with totally disorganized sarcomeres (resembling a fetal muscle fibre) occupies the upper one third of the field. In the middle of the field there is a myelinated motor nerve within a thin endoneurial sheath.

176A

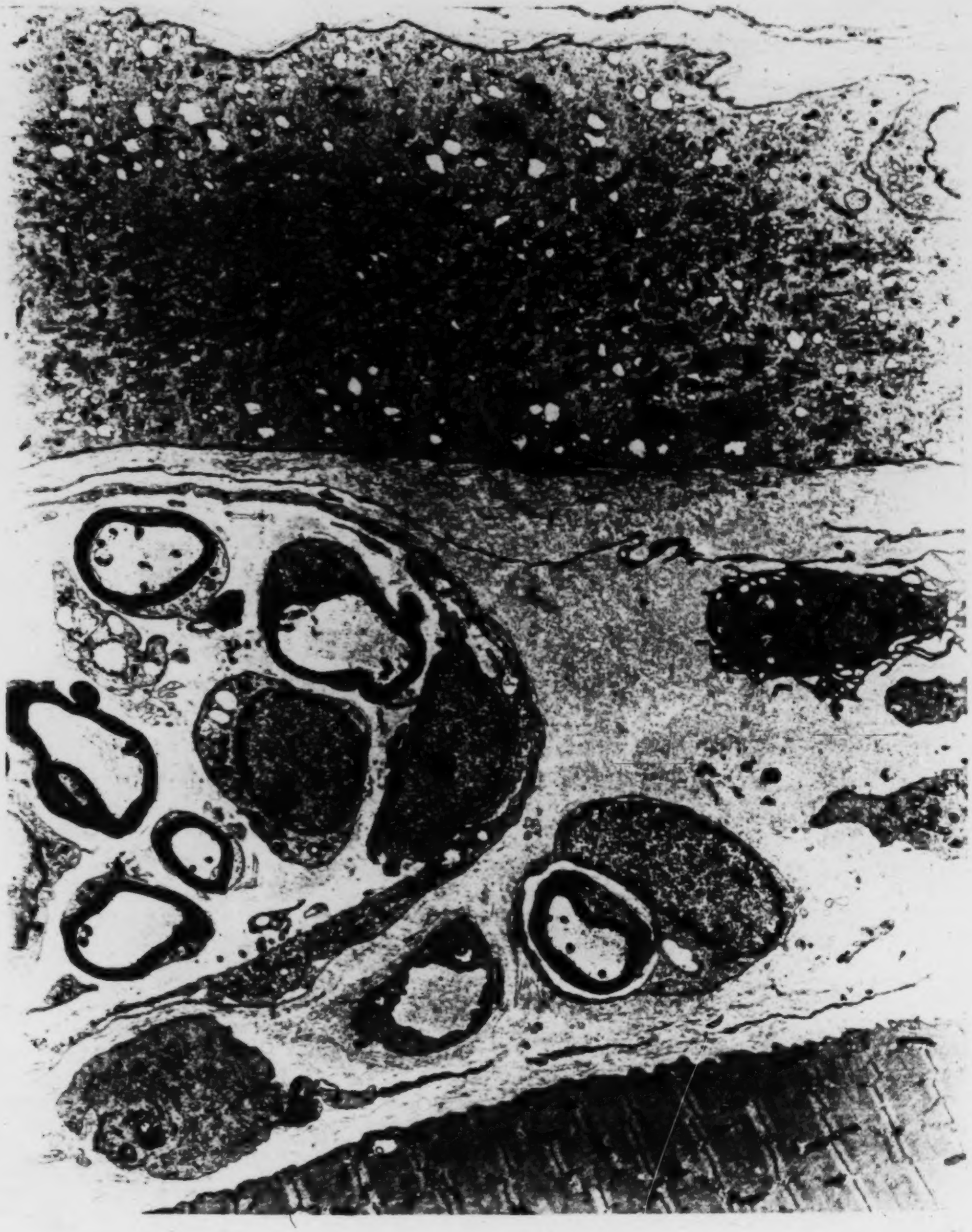


PLATE # 10 TWO DAYS POST-TRAUMA

Cross-section of motor nerve located in the endomysium containing five myelinated axons. At the top of the micrograph there is a mast cell with a large rounded nucleus and numerous large dense secretory granules.

177A

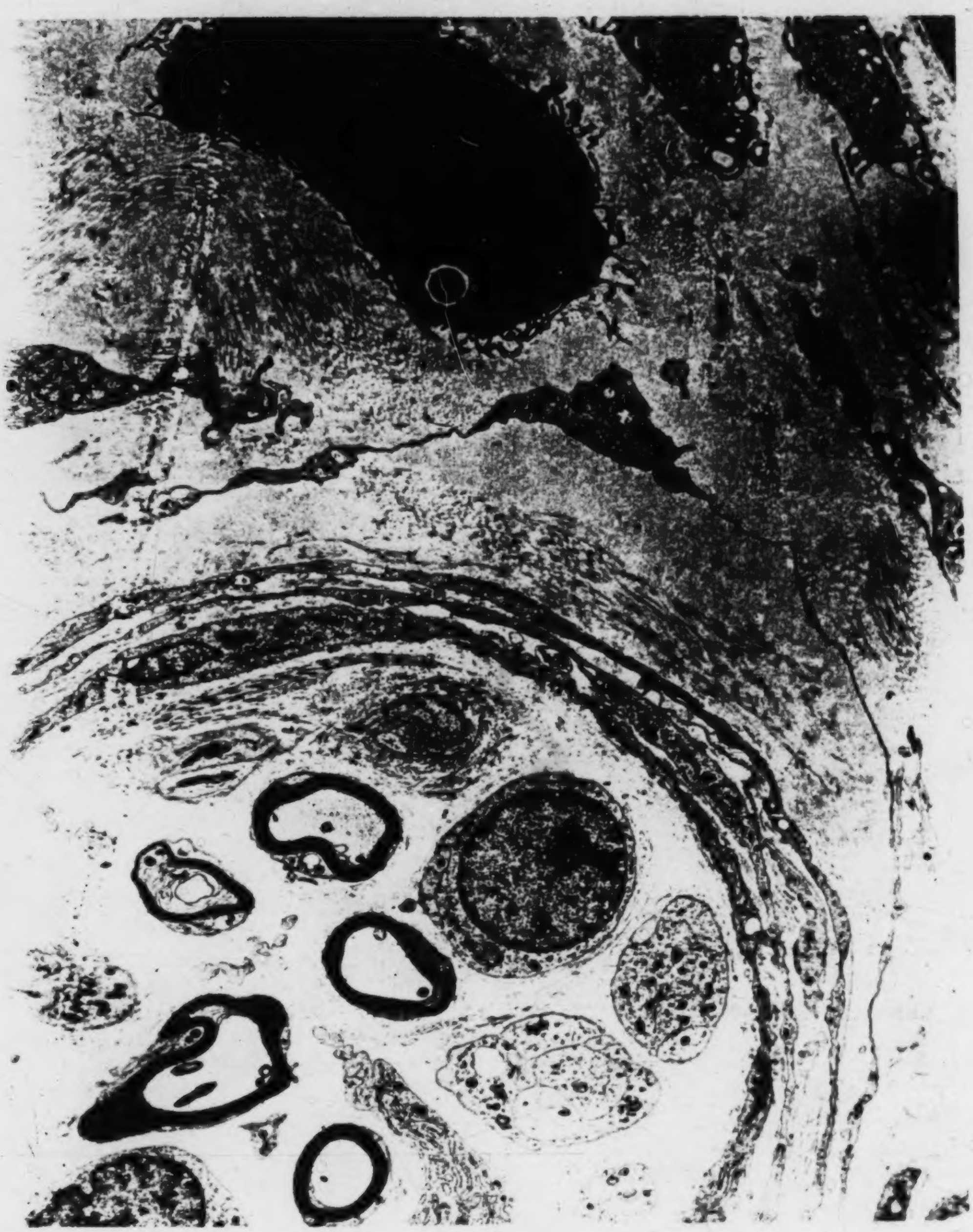


PLATE # 11 THREE DAYS POST-TRAUMA

Regenerating muscle fibre showing infolding of the plasma membrane, giving rise to an internalization of the stromal collagen.

178A

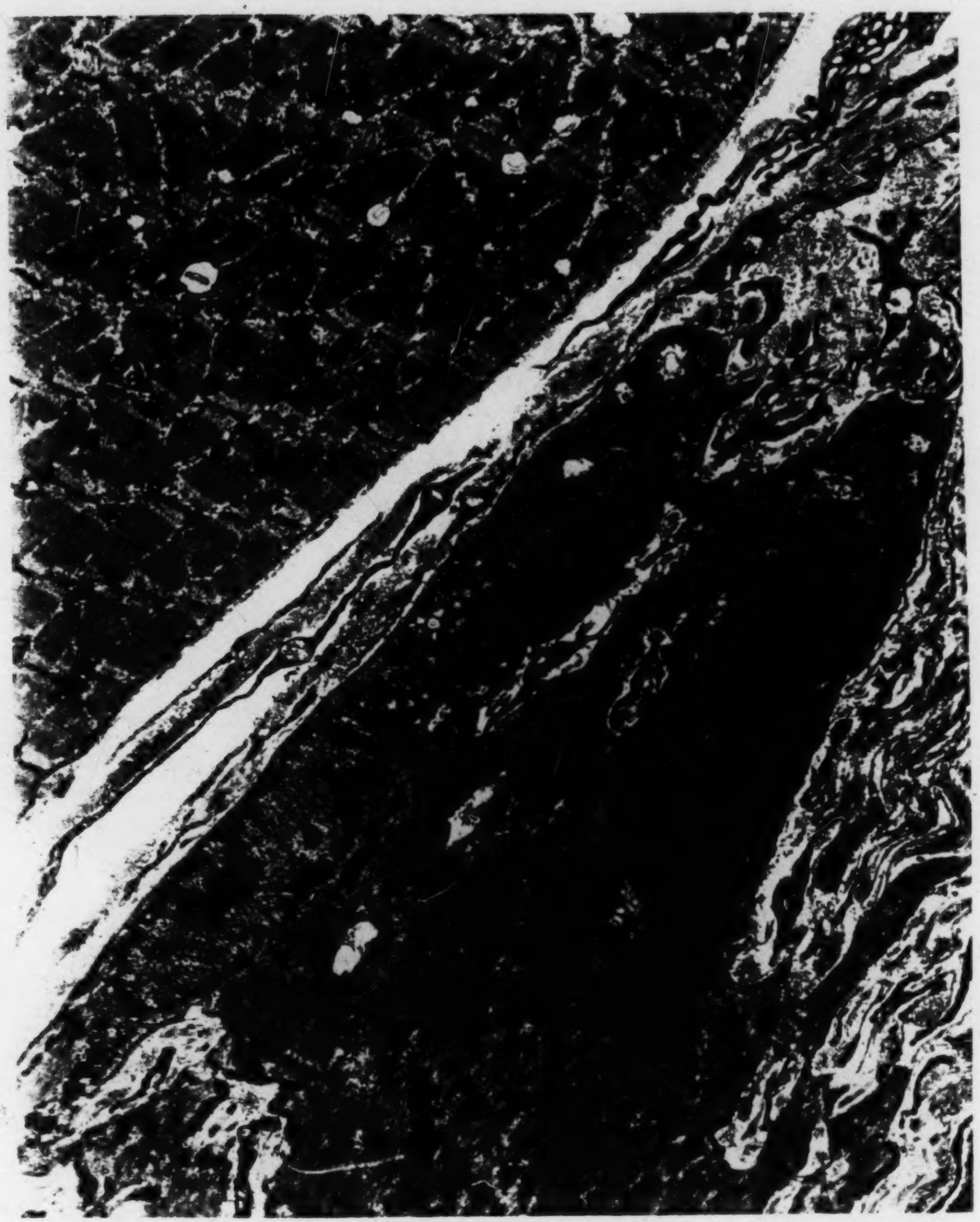


PLATE # 12 THREE DAYS POST-TRAUMA

Regenerating muscle fibre with infolding of the plasma membrane and internalized stromal collagen. A satellite cell lies inside the basement membrane of the muslce fibre. The chromatin of the satellite cell is marginated against the nuclear membrane.

179A

PLATE # 13 THREE DAYS POST-TRAUMA

Regenerating muscle fibre (similar to Figs. 18 and 19). A pair of satellite cells appear to lie outside of the basement membrane. Their cytoplasm contains numerous ribosomes and mitochondria.

180A

PLATE # 14 THREE DAYS POST-TRAUMA

This micrograph shows four nuclei in close proximity in a regenerating muscle fibre. Three of the nuclei have prominent nucleoli. There is an aggregation of mitochondria surrounding the nuclei.

181A

PLATE # 15 THREE DAYS POST-TRAUMA

Longitudinal section showing perimysial fibrosis. Fibrocytes are surrounded by dense colagenous stroma.

182A

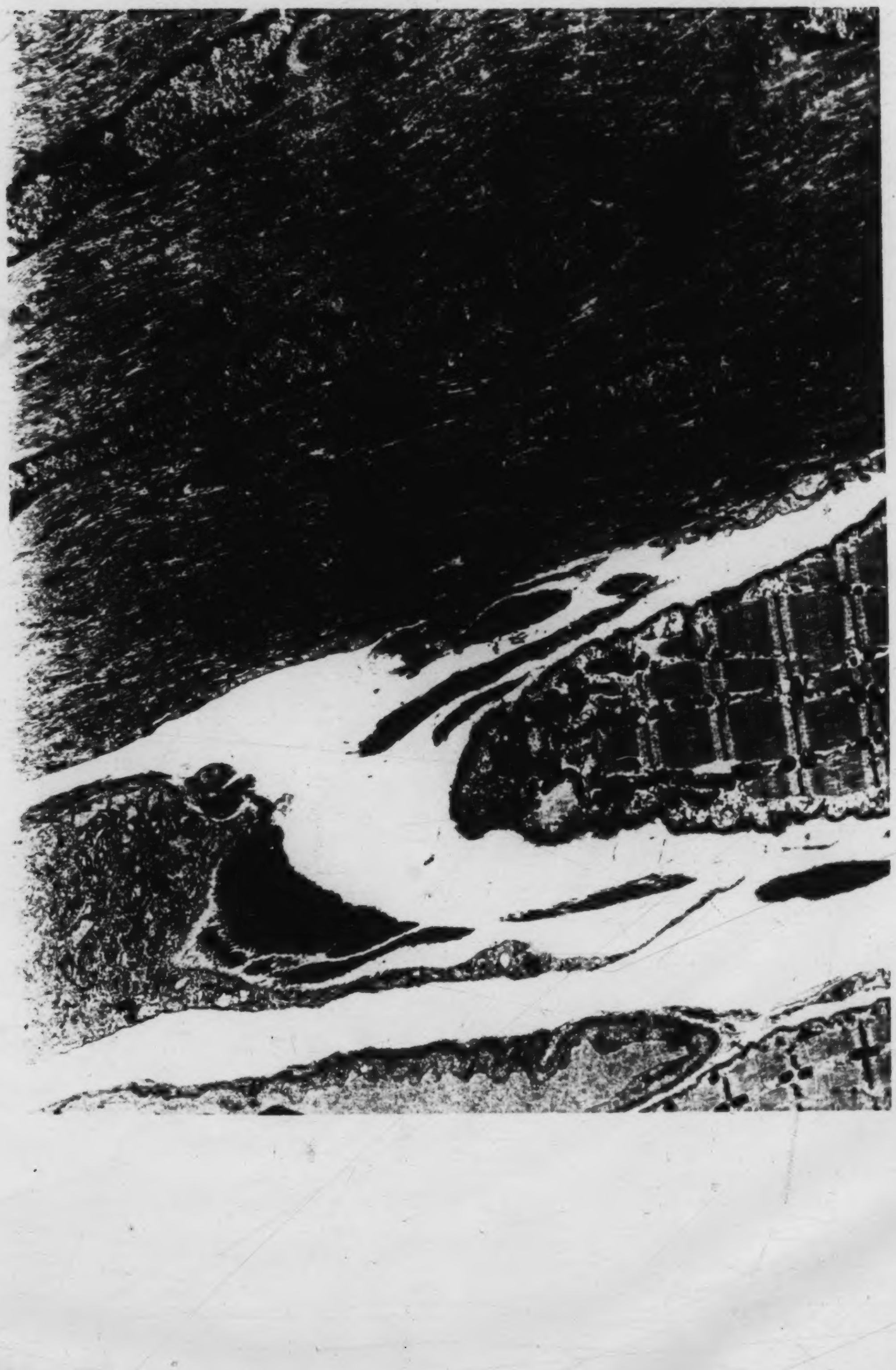


PLATE # 16 THREE DAYS POST-TRAUMA

Survey electron micrograph showing a regenerating muscle fibre with a highly folded sarcolemmal membrane (bottom centre), and portions of fibroblasts, macrophages and two capillaries within the adjacent endomysial stroma.

183A

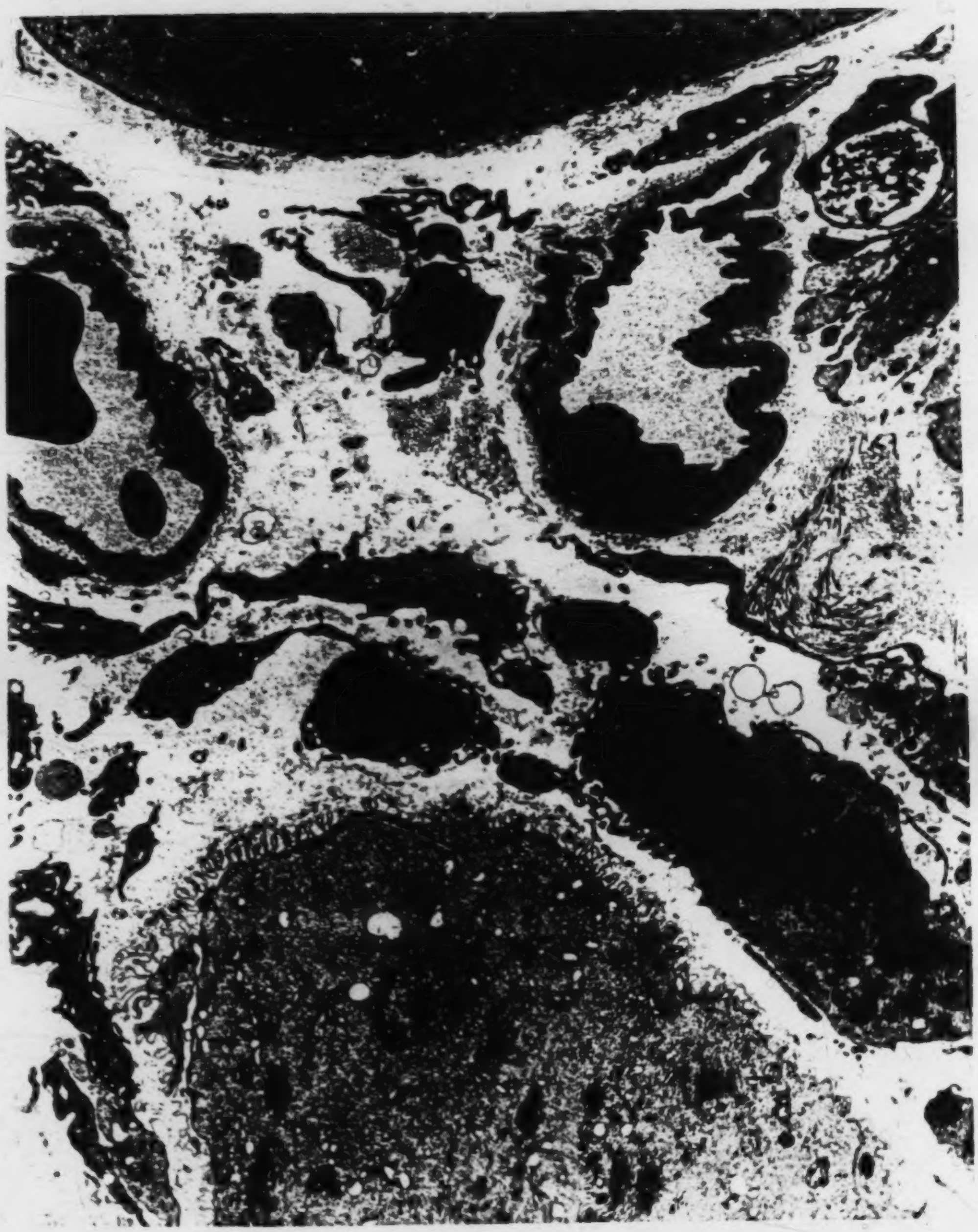


PLATE # 17 THREE DAYS POST-TRAUMA

Cross section of a normal arteriole within a focus of permysial fibrosis.

184A

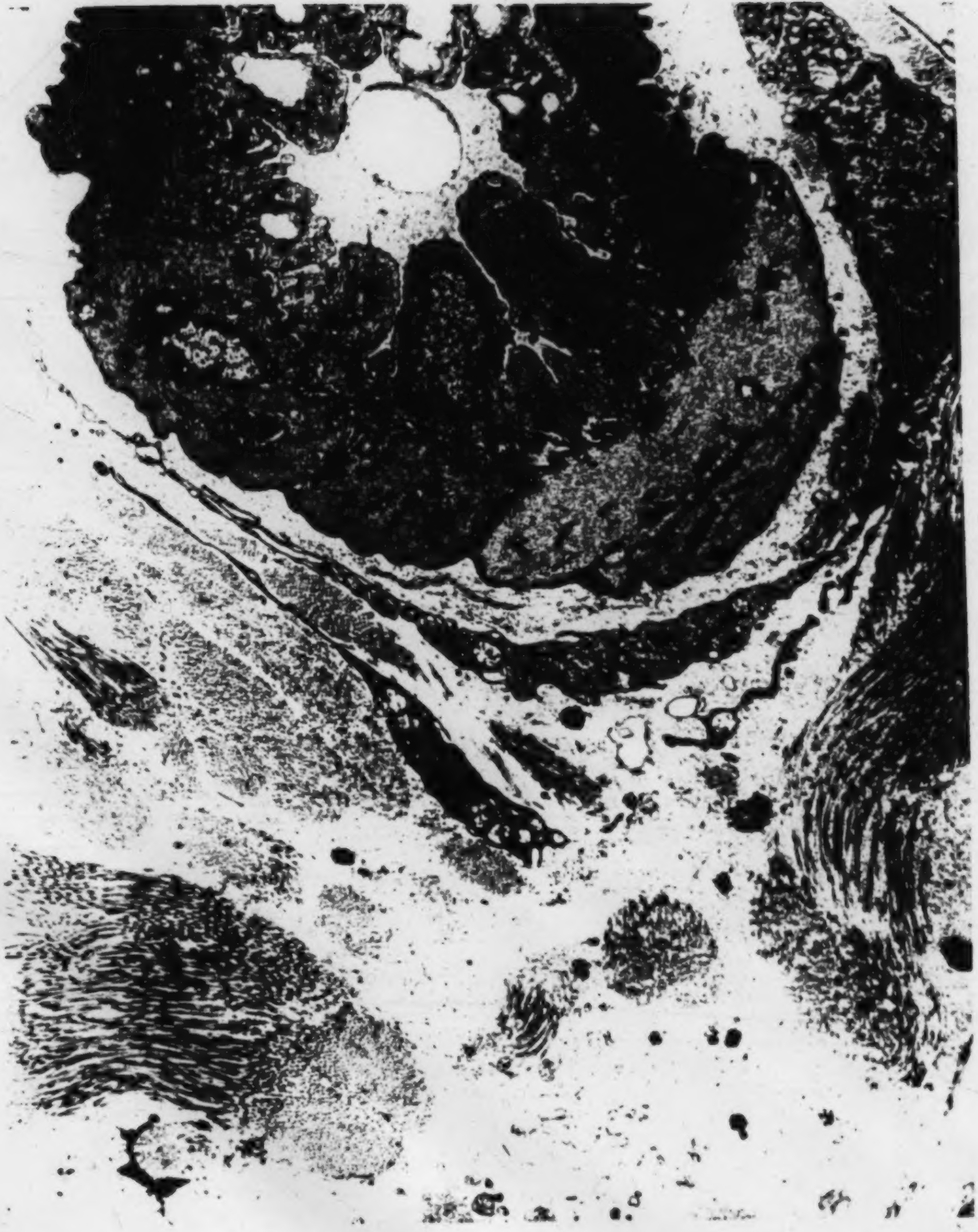


PLATE # 18 SIX DAYS POST-TRAUMA

This regenerating muscle fibre contains a cluster of three vesicular nuclei with prominent nucleoli. Large numbers of small mitrochondria surround the nuclei. Scattered throughout the sarcoplasm are bundles of myofibrils. the surrounding endomysium shows fibrosis.

185A